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SOIL SCIENCE

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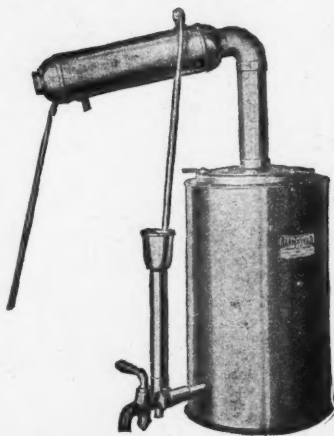
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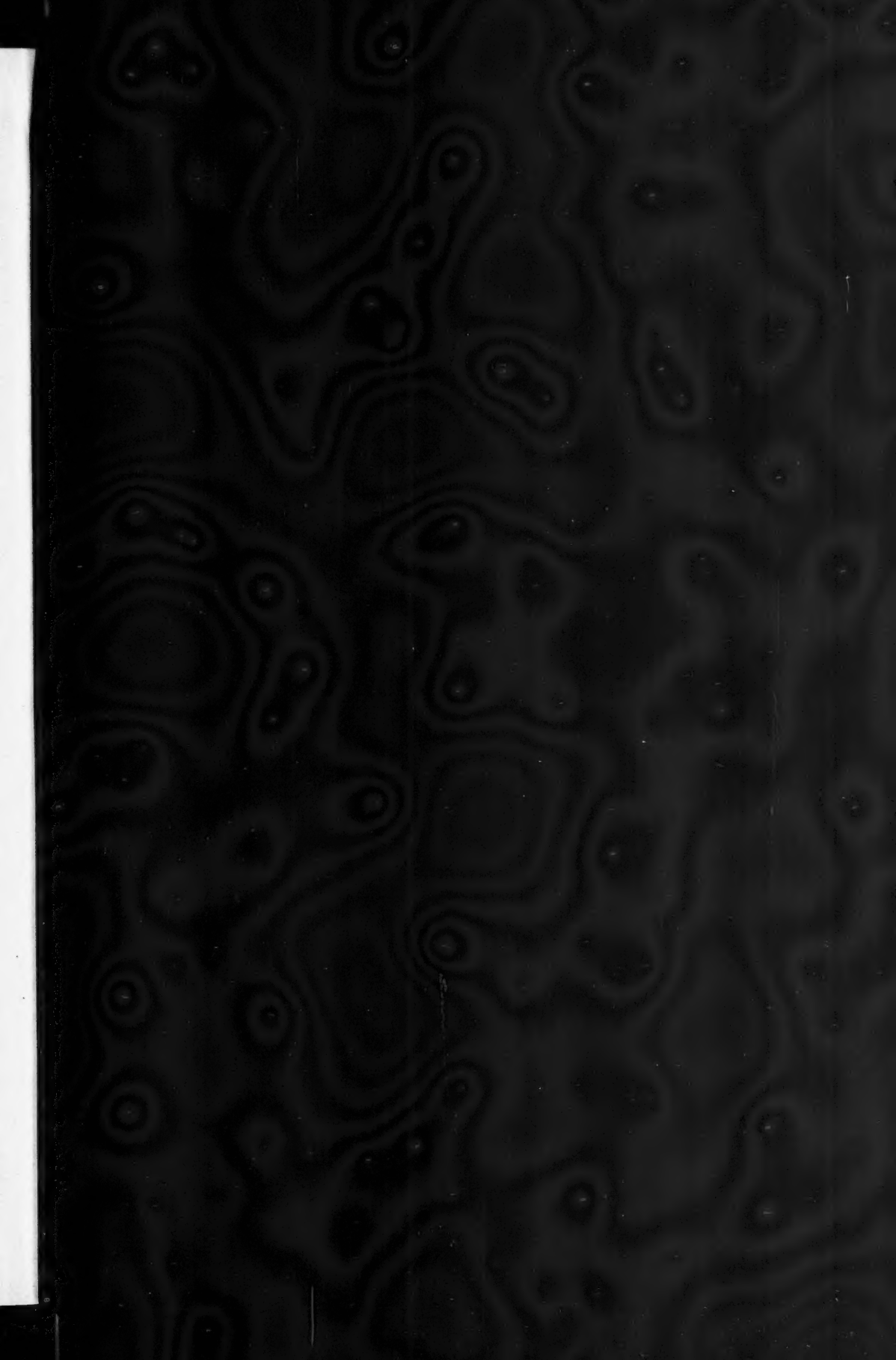
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ULTIMATE ANALYSIS OF THE MINERAL CONSTITUENTS OF A HAGERSTOWN SILTY CLAY LOAM SOIL AND OCCURRENCE IN PLANTS OF SOME OF THE ELEMENTS FOUND¹

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INTRODUCTORY

The soil for which analysis is here reported is being used in a 20-year cooperative experiment between the Departments of Horticulture and of Experimental Agricultural Chemistry. This experiment is being conducted in steel rims, forty-two in number, to determine the effect of fertilizers on the yield, growth, and other physiological functions of the apple tree. It is the result of inconclusive chemical studies conducted during 1917-19 on the soils and plants of the experimental orchards throughout the state. The variables have been eliminated in the present investigation as nearly as possible by using a uniform soil obtained by thorough and complete mixing of the sub-, subsurface, and surface-soil and also by means of a uniform stock. The soil with which these rims were filled was taken from a strip of virgin land adjacent to the experimental orchard, which lies about a mile and a half to the northeast of the well known general fertilizer plats, at the junction of the Stonehenge and Trenton formations, and is classed by Mooney, Shaw et al. (55), as a clay loam of the Hagerstown series. However, Shaw recognizes considerable variations in the texture and other physical qualities of these soils, e.g. he distinguished as present in the general fertilizer plats the following sub-classes: silty clay loam, heavy loam, clay loam and clay. The mechanical analyses (cf. table 1) of the soil in this experiment would indicate that it is a mixture of the Hagerstown silt loam and clay loam. This soil is probably formed by the disintegration of limestone rocks and represents that portion of the soil mass adjacent to the parent rock.

In an intensive experiment of this character it was regarded as fundamental to have as complete a knowledge not only of the total constituents of the soil

¹ Contribution No. 1 from the laboratories of the Department of Agricultural Chemistry of the Pennsylvania State College Agricultural Experiment Station.

² The mineralogical section of this paper (cf. p. 18) is based on the micro-petrographical examination made by Professor A. P. Honess. The author wishes to take this opportunity to express his indebtedness and gratitude for the guidance given him by the late Dr. William Frear with whom the author has been associated for nearly 10 years and under whose general direction the chemical phase of this project was initiated.

but also of the total amount of each element present, which can only be obtained by making a complete examination following the fusion method for rock analyses and adopting quantitative procedures of approved validity.

Robinson (62, p. 12-13) has reported the occurrence of a number of the rarer elements in a Hagerstown loam soil taken one mile northwest of Conshohocken, Pa., which is not far from metamorphic rocks. Fragments of mica schist (1.8 per cent of the stony fragments) occur in the surface soil. This showing is quite different from the conditions at State College, where the soil has been formed in place by weathering of the limestone belonging to the lower Silurian formations. The existence of these elements in this soil is conceivably susceptible of several explanations. If strictly residual then the origin would be the underlying limestone, but it is very questionable if these rarer elements would be found in the limestone underlying the soil. This point will be investigated later. As Merrill (54, p. 4-8) points out, the agencies which have been instrumental in the formation of soils have been so complex that many of our soils are but secondary rocks in a state of loose consolidation, and many of the accumulations classed as residual have been derived by disintegration in situ of alluvial materials which have been brought down years ago and deposited in shallow seas. Moreover, because of the transporting power of water, wind, or moving ice, few residual deposits have retained their virgin purity, but have become more or less contaminated with materials from near or distant sources.

Dr. E. S. Moore, Dean of the School of Mines of the Pennsylvania State College is of the opinion that these rarer elements have, probably, been derived "from the extensive pre-Cambrian area, known as the Appalachia, which existed to the east and northeast of this region at the time these Palaeozoic rocks were being laid down."

THE ISOLATION OF THE RARER ELEMENTS IN THIS RESIDUAL SOIL

Clarke (11, p. 15-22), Merrill (54, p. 312), Washington (75), and Hillebrand (42, p. 25-30) have been consulted in obtaining the following data on the soil used in this study:

Aluminum: Found chiefly in silicates such as the feldspars and the micas. It occurs in this soil in the mixed feldspars, $R'AlSi_3O_8$, in which R' may be Ca, Mg, Na or K; mica, $H_2(KNa)Al_2(SiO_4)_2$; tourmaline, $R'_3Al_3B_3(OH)_3Si_6O_{18}$, in which R' may be Fe, Mg or alkali metals; and chlorite, $H_2(Mg,Fe)_2Al_2(SiO_4)_2$.

Barium: Widely distributed in small quantities throughout igneous rocks. Professor Honess has found barite in sediments near Bellefonte, Pa., and has called the attention of the writer to the statement by Dana (19) that it has been proven to exist in amounts varying from 0.45 per cent to 2.20 per cent in orthoclase and some acid plagioclases, which are probably the original sources of barium, from which it is dissolved. It occurs also as carbonate and witherite. Failyer (27) reports that barium is present in most soils throughout the United States.

Boron: An essential constituent of several silicates. It is present in this soil in tourmaline, which has been found by Doctor Moore in the Tuscarora sandstones and quartzites.

Caesium: A rare metal—the rarest of the alkalis. Often found in lepidolite, $(Li,K)_2(FOH)_2Al_2SiO_5$; Vernadski (69, 70) found spectroscopic traces in the feldspars and micas, which are the probable sources of the element in this soil.

Calcium: An essential constituent of many rock-forming minerals such as the amphiboles and the pyroxenes. It occurs as dolomite, $CaMg(CO_3)_2$, and amphibole, $Ca(Mg,Fe)_2(SiO_4)_2$. Sericite and the mixed feldspars found in this soil contain small amounts of calcium.

Chromium: Very widely diffused in the form of chromite, $(Fe,Cr)[(Cr,Fe)O_2]_3$, and especially in ferro-magnesian rocks. Its source in this soil is probably chlorite.

Fluorine: Fluorine is found in a number of silicates such as tourmaline and the micas, which are its probable source in this soil.

Iron: Found in practically all rocks. Occurs as limonite, $H_2Fe_2O_4(H_2O)_4$; ilmenite $FeTiO_3$; magnetite, Fe_3O_4 ; and also chlorite and amphibole in this soil.

Lithium: Traces are found in nearly all igneous rocks. The most important lithia minerals are lepidolite, $(Li,K)_2(F,OH)_2Al_2Si_2O_6$; spodumene $LiAl(SiO_3)_2$; and the lithia tourmalines and alkali feldspars. Washington (75, p. 20) states that it frequently occurs in rocks high in sodium.

Magnesium: Magnesium occurs in this soil as amphibole, dolomite and chlorite. The micas also contain magnesium.

Manganese: Found in most rocks and soils in small amounts in the ferro-magnesian minerals. Through alteration it appears sometimes on the surfaces of limestones and sandstones. Its probable source in this soil is chlorite.

Molybdenum: Hillebrand (42, p. 185) has found it in minute quantities in siliceous rocks. It has also been found in granite in the form of molybdenite, MoS_2 , but its source in this soil has not been determined.

Phosphorus: Found in nearly all igneous rocks in the mineral apatite. Its source in this soil has not been determined unless it occurs in the limonite (cf. p. 11).

Potassium and sodium: These metals are present in this soil in the mixed feldspars and micas.

Rubidium: The source of this metal in this soil has not been determined. It is found in lepidolite, $(Li,K)_2(F,OH)_2Al_2Si_2O_6$, and in some mineral springs. It is reported as being present in the waters of the Caspian Sea.

Strontium: This is a common ingredient of igneous rocks in small amounts. The most important minerals are celestite, $SrSO_4$, and strontianite, $SrCO_3$. Lesley (47) reports the absence of celestite in the beds of this limestone formation. Professor Honess, however, has called to the author's attention the fact that Dana (20) states that it occurs in the limestone regions of Central Pennsylvania, at Bells Mills, Blair County. It has recently been found by Mr. A. H. Dewey of the School of Mines of this Institution at Williamsburg, Pa.

Sulfur: The source of sulfur in this soil has not been determined. It certainly exists as sulfates and in organic combination. No sulfide minerals were found.

Titanium: Titanium is almost invariably present in igneous rocks and the sedimentary material derived from them. It occurs in this soil as ilmenite, $FeTiO_3$, and rutile, Ti_2TiO_4 .

Vanadium: This is diffused with titanium through all primitive granite rocks, has been found in rutile and bauxite, and is reported as occurring very commonly in small amounts in residual limestone deposits, which is probably the source of the vanadium found in this soil. Phillips (58) suggests that vanadium in sedimentary rocks may be derived from the decay of mineral organisms—the acidians.

Zirconium: This is allied to titanium and rather widely diffused in the igneous rocks and has been found by Doctor Moore in the Tuscarora sandstones and quartzites. Hillebrand (42, p. 25-30) and Robinson (62, p. 3) state that it occurs in largest amounts in soils high in silica and sodium.

The present investigation is of special interest because of the isolation by quantitative methods of known validity and confirmatory qualitative tests of the rarer elements vanadium, chromium, molybdenum, zirconium, titanium, rubidium, caesium, lithium and also of the elements barium and strontium, concerning the presence of which in this soil series much doubt has existed.

THE DISTRIBUTION OF THE ELEMENTS PRESENT IN THIS SOIL MECHANICAL ANALYSIS

The mechanical analysis was carried out by the method described by the Bureau of Soils (8, p. 7-24) on samples prepared according to the methods

prescribed by the Association of Official Agricultural Chemists (2) dried at 110°C. Duplicate determinations were made and are shown in table 1.

It is of interest to note the difference in mechanical composition of this soil and that of the grass lands of the general fertilizer plats as given by Frear and White (30), in table 2.

The soil used in the apple rim experiment is, therefore, of a much more clayey texture than the major portion of the land of the college farm, though the relatively larger amount of clay present is not sufficient to interfere with the ease of tillage, excellence of drainage and formation of clods on drying, which are characteristic of this soil type.

TABLE 1

GRADE	SURFACE SOIL TO 9.5 IN.		SUBSURFACE SOIL 9.5-18.5 IN.		SUBSOIL 18.5-42 IN.	
	(a)	(b)	(a)	(b)	(a)	(b)
	per cent	per cent	per cent	per cent	per cent	per cent
Coarse sand, 1.0-0.5 mm.....	7.30	7.52	5.45	5.43	4.43	4.45
Medium sand, 0.5-0.25 mm.....	4.82	5.20	3.90	3.74	3.51	3.40
Fine sand, 0.25-0.1 mm.....	3.40	3.40	3.07	3.00	3.05	3.01
Very fine sand, 0.1-0.05 mm.....	12.80	12.51	14.00	13.98	12.22	12.50
Silt, 0.05-0.005 mm.....	50.30	50.52	45.43	46.00	46.45	47.44
Clay, 0.005 mm. or less.....	15.78	15.67	23.29	22.76	25.57	24.33
Loss on ignition.....	5.30	5.30	5.00	5.00	5.50	5.50
Total.....	99.70	100.12	100.14	99.91	100.73	100.63

TABLE 2

GRADE	SURFACE SOIL TO 7 IN.	SUBSURFACE, 7-14 IN.
	per cent	per cent
Very fine sand.....	6.44	6.78
Silt.....	75.05	67.83
Clay.....	9.45	17.98

Samples were taken from each wheelbarrow load as the rims were being filled. These were placed in cans and stored as "surface-", "subsurface-" and "sub-" soil. The samples from each of these three layers were treated as follows: The whole soil sample, about 270 kgm., was placed upon heavy paper, thoroughly mixed by turning over with a hand scoop, and divided into four segments in the usual way. The process of mixing and quartering was continued until the quantity of soil was reduced to about 2 kgm.

The three samples resulting were air-dried, washed and sieved as prescribed by the method of the Association of Official Agricultural Chemists (2). About 50 gms. of each sample, was finely ground in an agate mortar until all passed through a two hundred mesh silk bolting cloth.

THE CHEMICAL ANALYSIS

The complete chemical analysis of the soil, in general as outlined by Hillebrand (42), is given in table 3.

The analysis of the various soil fractions has not been undertaken. Fail-
 yer, Smith and Wade (28) have shown that as a general rule the smaller particles of soils are richer in potassium, calcium, magnesium and phosphorus than

TABLE 3

CONSTITUENT	SURFACE SOIL TO 9.5 IN.		SUBSURFACE SOIL 9.5-18.5 IN.		SUBSOIL 18.5-42 IN.	
	(a)	(b)	(a)	(b)	(a)	(b)
	per cent	per cent	per cent	per cent	per cent	per cent
SiO ₂	72.320	72.340	67.720	67.620	63.230	63.000
Al ₂ O ₃	10.192	10.322	13.503	13.667	15.994	15.974
Fe ₂ O ₃	3.710	3.670	5.580	5.580	6.840	6.840
FeO.....	0.540	0.550	0.350	0.350	0.207	0.207
V ₂ O ₅	0.036	0.036	N E	N E	0.090	0.080
Cr ₂ O ₃	0.002	0.002	N E	N E	0.002	0.002
MoO ₃	P*	P	N E	N E	P	P
TiO ₂	0.340	0.320	0.480	0.460	0.440	0.480
P ₂ O ₅	0.102	0.095	0.102	0.098	0.077	0.078
ZrO ₂	0.037	(0.037)†	0.045	(0.045)	0.050	(0.050)
MnO.....	0.240	0.230	0.250	0.260	0.230	0.260
CaO.....	0.660	0.700	0.650	0.660	0.900	0.910
MgO.....	0.650	0.680	0.980	1.050	1.370	1.350
BaO.....	0.009	(0.009)	0.019	0.020	0.019	0.019
SrO.....	0.016	0.010	0.022	0.018	0.033	0.028
Rb.....	0.001	0.001	N E	N E	0.001	0.001
K ₂ O.....	3.960	3.890	4.220	4.180	4.420	4.400
Cs.....	N F	N F	N E	N E	P	P
Na ₂ O.....	2.280	2.240	1.280	1.300	1.350	1.280
Li.....	Trace	Trace	Trace	Trace	Trace	Trace
Cl.....	Trace	Trace	Trace	Trace	Trace	Trace
S.....	0.062	(0.062)	0.051	(0.051)	0.062	(0.062)
SO ₃	0.017	0.017	0.020	0.020	0.017	0.017
Loss on ignition.....	5.300	(5.300)†	5.000	(5.000)	5.500	(5.500)
Total.....	100.474	100.511	100.272	100.379	100.832	100.538
Less oxygen equivalent of S.....	100.448	100.485	100.250	100.357	100.806	100.512

* P = present; N E = not examined; N F = not found.

† Parentheses indicate that duplicate determinations were not made.

the coarser particles and the concentration becomes greater the greater the weathering, and that the larger mechanical fractions contain these elements in forms which by protracted weathering will become more soluble and will ultimately be concentrated in the finer compounds.

The mineralogical examination of this soil (cf. p. 10) confirms these conclusions.

NOTES ON THE CHEMICAL ANALYSIS

1. Before mixing with sodium carbonate prior to the fusion, the charge was ignited to destroy organic matter.

2. The silica not obtained by the fusion with HCl, after three evaporations and intervening filtrations (41), was separated from the mixed oxides, of alumina, etc., by fusion with potassium bisulfate and added to the weight of the silica already obtained. The weight of silica was corrected for impurities by evaporation in HF and H₂SO₄.

3. The precipitation of iron and aluminum was made with ammonia by Blum's procedure (6). The author found no manganese, alkaline earth metals or magnesium in this precipitate.

4. Manganese was determined by the ammonium sulfide method. The amount of manganese that escaped precipitation was negligible, for none was found by the colorimetric method in the magnesium pyrophosphate precipitate in which, if any was present, it would occur.

5. The results for ferrous iron are to be regarded as approximate. The various devices used to prevent oxidation of the FeO to Fe₂O₃ are fully discussed by Hillebrand (42, p. 189-192) and Washington (75, p. 122-126). The presence of organic matter, moreover, might reduce some of the ferric iron during the operations. Cooke's method (15) simplified by Barneby (3) was adopted.

6. Nickel, cobalt, copper, zinc, mercury, silver, tin, lead and arsenic were not found.

7. Calcium and strontium were precipitated as oxalates and separated, after conversion into the nitrates, by the ether-alcohol method (32). The strontium was weighed as the sulfate, which was examined by the spectroscope.

The orange α line of wave length 6060 and the blue δ line of wave length 4607.5 identified Sr. The band spectrum of calcium with characteristic line of wave length 4226.9 was also observed. This examination confirms the presence of strontium in this soil, though complete separation from calcium was not effected. It is remarkable that, though barium was absolutely identified, no barium lines were observed in this separate, showing complete separation from this element.

8. Barium together with zirconium, total sulfur and rare earths were determined by fusion with sodium carbonate and a little potassium nitrate on a separate 2-gm. charge of the soil. The zirconium and rare earths were separated from barium by means of dilute warm sulfuric acid. After evaporating the residue remaining undissolved with hydrofluoric acid and sulfuric acid, it was freed from calcium and strontium by repeated fusions with sodium carbonate, solution of the melts in water and reprecipitation with sulfuric acid. The barium sulfate obtained in this manner was dissolved in concentrated sulfuric acid and reprecipitated by water to remove the last traces of calcium. The barium sulfate thus obtained was ignited, weighed and examined by the spectroscope.

The green barium line of wave length 5535.7 was used for identification. Separation from calcium and strontium was completed, as none of the characteristic lines of these elements were present.

9. The method which Robinson (62, p. 7; 64), used to estimate the amounts of rubidium and caesium was adopted. Ten-gram portions of soil were used. The method removes a large part of the potassium and sodium chlorides present by fractional precipitation with platonic chloride and then by strong hydrochloric acid. The platinum was removed from the double platonic chlorides Cs₂PtCl₆ and Rb₂PtCl₆ by Horsch's method (44). After filtering and evaporation, the weights of the mixed chlorides from the surface and subsoil were 0.8 and 0.2 mgm. respectively. After treatment with hydrochloric acid, it was filtered into small vials and examined by means of the spectroscope, using the flame spectrum, according to the method of Gooch and Phinney (37).

The blue rubidium lines of wave length 4215.6 and 4202 were clearly defined in both the case of the surface and subsoil. In the case of the subsoil, but not of the surface soil, a doublet of wave length 4593.3 and 4555.4 was plainly discernible.

10. The examination for lithium was made on the solution obtained after removal of the potassium as potassium platonic chloride from the mixed chlorides of potassium and sodium. The platinum was removed by Horsch's method. The lithium lines of wave length 6708.2 and 5104 were observed.

An attempt was made to effect a separation of the lithium and sodium by Gooch's method (36), but the amounts of lithium chloride obtained were too small to obtain pure.

11. Calcium was always found in the magnesium pyrophosphate and was removed by treatment of the latter with dilute sulfuric acid and alcohol. The calcium sulfate obtained was filtered, weighed, and suitable corrections made in the figures for calcium and magnesium oxides.

12. The zirconium, rare earths, and total sulfur were determined by fusing 2-gm. portions of the soil powder (see note 8).

The zirconium pyrophosphate obtained by ignition of the phosphate was freed from any titanium that might possibly be present by fusion with sodium carbonate, leaching with water, fusion of the residue with potassium pyrophosphate, solution of the melt in 20 per cent sulfuric acid [Recolardot and Reglade (61)] to prevent contamination with titanium, iron, and chromium with the addition of hydrogen peroxide and reprecipitating with sodium hydrogen phosphate. The precipitate was ignited and weighed as the pyrophosphate.

Certainty as to its identification was again established by fusing with sodium carbonate, leaching, igniting the residue, fusing with potassium pyrophosphate and reprecipitating with ammonia, filtering, igniting and weighing as ZrO_2 . This was then dissolved in concentrated sulfuric acid, precipitated with ammonia, dissolved in hydrochloric acid and tested with tumeric paper after evaporation almost to dryness.

13. The rare-earth metals were sought in the filtrate from the zirconium phosphate by precipitating with KOH and treating the precipitate with HF, converting into sulfates, precipitating with ammonia, and finally converting into the oxalates. The amounts were extremely small and no concordant results could be obtained.

14. Vanadium, chromium and molybdenum were determined by the Hillebrand (42, p. 185) method on a 5-gm. charge of the soil powder.

Confirmatory qualitative tests for the presence of vanadium and molybdenum were applied as follows: The solution was evaporated to dryness and heated to expel excess of sulfuric acid. The residue was taken up with 2-3 cc. of water containing a few drops of dilute nitric acid. On addition of a few drops of hydrogen peroxide a characteristic brownish tint developed. Certainty as to the identity of molybdenum was established by igniting the sulfide in porcelain and adding a single drop of concentrated sulfuric acid. On heating, a beautiful blue color indicated the presence of molybdenum.

15. The literature on the determination of sulfur as barium sulfate is voluminous. The total sulfur was determined by fusion with sulfur-free sodium carbonate and about 0.5 gm. potassium nitrate, solution of the melt in water, reduction of the manganese with ethyl alcohol and precipitation as barium sulfate. In connection with this determination it is interesting to note that Hillerbrand has shown, contrary to the experience of Stoddart (67), that it is unnecessary to remove the silica. For, if the filtrate measures about 100-200 cc. and is acidified only slightly with hydrochloric acid in the cold, no separation of the silica occurs. This is indeed fortunate for in the case of soils the amount of silica is so high that filtration from the silica would be an almost impossible task. The writer found absolutely no separation of silica by this method. The sulfur given in the tabulated statement was obtained by subtracting from the total sulfur that present as SO_3 , obtained by treating with 100 cc. of 16½ per cent HCl.

16. The loss on ignition has no particular significance. It represents the algebraic sum of a number of changes depending on the temperature employed. With increasing temperature the sulfates are decomposed, all the sulfur being driven off, at which point the alkalies begin to escape. The figures, which are the average of a large number of determinations, are to be regarded as approximations only, for it is difficult to prevent reduction by the organic

matter, even if a low temperature with proper precautions is employed. This fact together with the difficulty of dehydrating the hydrated minerals like mica, limonite and chlorite accounts for the summation being over 100.

17. Boron and fluorine exist in tourmaline and mica respectively, both of which have been found in this soil.

The author's attempts at a quantitative estimation of these elements in this soil have not met with much success. Cook (12) and Cook and Wilson (13, 14), have described without giving details the methods used in estimating boron in soils. The author, however, could not obtain consistent results with the colorimetric method used by these authors or by the method outlined by Hillebrand (42, p. 234-238). The objection found to the colorimetric method was the inability to secure reagents used in the test that gave no color with filter paper dipped in a 0.2-per cent solution of curcumin. The amount present was too small to obtain any success with Hillebrand's method.

18. Two, and sometimes three precipitations were carried out throughout this work. Wherever practicable evaporations and precipitations were made at the boiling point in platinum dishes. The filtrations were made into pyrex glass beakers. Blank determinations were made on all reagents.

DISCUSSION OF THE ANALYSIS

Examination of the results indicates that the surface and subsurface soil contain more phosphoric acid than the subsoil. Sodium is much higher in the surface soil than in the other two layers, whereas the subsoil is richest in calcium, magnesium and potassium.

The tendency of iron to work its way down is manifest, the subsoil containing almost twice as much Fe_2O_3 as the surface soil. Inasmuch as the FeO in the surface soil is more than double the amount in the subsoil, it would appear that the reduction effect due to the larger amount of organic matter in the surface soil offsets the oxidizing effect due to aeration.

The presence of aluminum and iron in large amounts favors the formation of insoluble phosphates of these elements. This may explain why phosphorus is the limiting factor in these soils.

Manganese remains about constant in the surface, subsurface and subsoil.

Titanium oxide ranges from 0.4 per cent to 1.5 per cent in the chief soil types in this country; it is, therefore, relatively low in this soil.

It would appear that the sulfur present is sufficient for present needs; but much of it is combined in such a state, probably in organic combinations, that is not readily soluble in acid.

There is no evidence of the presence of sulfides such as pyrite or marcasite.

It is remarkable that lead and zinc, found in a number of localities in both Siluro-Cambrian and later limestone formations, were not present even in traces in this soil.

Analysis of more or less disintegrated dolomite by Knight (46) showed that 10 per cent of the CaCO_3 had been removed, and that the MgCO_3 had remained relatively stable. If the Ca and Mg in this soil had been derived entirely from dolomite we would have a ratio of $\text{CaO/MgO} = 30.4/21.7$. It is obvious from the results of the chemical analysis that a large portion of the calcium

must be derived from amphibole, and that a considerable portion of the magnesium is derived from chlorite.

The amounts of chlorine and sulfuric acid are low. If, as experimental evidence seems to indicate, these radicals are necessary for the synthetic processes in plants or as carriers of the alkalies from the soil to the plant, fertilization with chlorides and sulfates may have considerable effect in this experiment.

This soil is naturally rich in potash, and inasmuch as the orthoclase fragments were found by the micro-petrographical determination to be highly kaolinized and broken up, this may explain the reason why potash dressings alone cause little or no crop increase on this soil type.

THE MINERALS OF THIS SOIL

Chamberlain and Salisbury (9) and Delage and Lagatu (22, 23) were the first to show that nearly all the common rock-forming minerals are to be found in any ordinary soil, formed in the process of weathering and presenting clear unaltered faces. They recognized, also, the result of metamorphic changes, especially in the feldspars, where alteration products could be recognized in the mineral fragments.

The chemical analysis indicates the total amount of elements present in a soil; the mineralogical analysis, however, helps to show in what way these elements are combined.

Considerable work has been done on the solubility of minerals such as the feldspars, micas, tourmaline, chlorite, orthoclase, muscovite, dolomite, limestone, rutile, quartzite, etc., all of which are measurably soluble in water. For details the reader is referred to the work of W. B. and R. E. Rogers (65), Skey (66), Beyer (4), Daubree (21), Clarke (10, 11, p. 532), Cushman (16, p. 6-7) and Funk (33). Cushman's work is of interest as showing that fresh particles of siliceous rock form, upon contact with and decomposition by water, a colloid or gelatinous substance that forms a film on contact with their surfaces which possesses great power of adsorption.

THE AVAILABILITY OF SOME OF THE MINERALS PRESENT IN THIS SOIL

The experimental results on the availability of the various minerals in this soil are not clearly defined.

Cushman (17) called attention to a series of experiments with tobacco seedlings which showed that finely ground orthoclase was nearly as efficient a source of potash plant food as the more soluble potash salts. He emphasizes the necessity for improvements in methods and machines for grinding rock. Prianshnikov (59), Fraps (29, p. 5-16) and Bieler-Chatelan (5) conclude from their experiments that plants assimilate very little potash from orthoclase and microcline even when finely powdered. Fraps showed, however, that they removed from 7 to 13 per cent of the potash from the finely ground micas, biotite and muscovite and Prianshnikov's experiments indicated an assimilation by plants of about 17 per cent of the potash from this mineral. Breazale and Briggs (7) showed that the availability to plants of potash in soils derived from orthoclase-bearing rocks is not increased by addition of lime or gypsum.

The author has been unable to find any experimental work on the availability of the other minerals present in this soil.

THE MINERALOGICAL EXAMINATION

The micro-petrographical examinations were made by Professor Arthur P. Honess of the Department of Geology of the School of Mines of the Pennsylvania State College. Examinations were made on separates made by mechanical analysis by the writer. Professor Honess states that owing to the lack of necessary equipment the examination has not been as complete as could be wished.

Surface Soil

In the surface soil the sand fractions were sieved and examined separately.

Very fine sand (0.1 mm.-0.05 mm.). Mixed feldspars about 50 per cent. Quartz about 35 per cent. The microcline occurs in blue-gray cleavage fragments usually fresh and unaltered, although fragments appear at times slightly kaolinized. The remaining 15 per cent represents iron oxides (some specimens of which are magnetic), rutile, chlorite, dolomite and tourmaline, with limonite predominant.

Fine sand (0.25 mm.-0.1 mm.). Quartz is present in larger amounts than feldspar. Many fragments of quartz from this sample are highly colored by iron oxide and frequently enclose particles which are brown to black in color and sometimes strongly magnetic. This is probably magnetite or ilmenite. The accessory species are the same as in the very fine sand.

Medium sand (0.5 mm.-0.25 mm.). This does not materially differ from the fine sand, except that quartz and dolomite are somewhat more abundant. There is not the variety of accessory species noted in the finer grades.

Coarse sand (1 mm.-0.5 mm.). This fraction is composed of about 85 per cent quartz. It occurs as rounded or subangular grains; but are occasionally almost spherical. Iron oxide is present either as independent grains or as patches adhering to quartz. A search was made for accessories by crushing part of the sample and mounting in canada balsam; no new species, however, were observed. The total absence of feldspars is noteworthy.

Subsoil

Sand. The sand fractions of the subsoil were not sieved into the various grades as in the case of the surface soil, but were examined as a whole. About 40 per cent consisted of quartz and 45 per cent of feldspar. Occasionally fragments of dolomite were found; limonite grains are common and also iron stained quartz. Some of the microcline fragments are well preserved, others are slightly colored by iron and show some slight evidence of alteration. As accessory components: Tourmaline, mica and rutile were observed.

Surface, subsurface and subsoil

Silts. The silts were difficult to determine due to their finely divided state. Some attempt has been made to determine the minerals present; complete analysis has not been possible because of the lack of necessary apparatus. A preponderance of feldspar (microcline) was noted with a little quartz and a variety of accessories among which tourmaline, chlorite, amphibole, and sericite were unmistakably present.

The silts from the surface, subsurface and subsoil do not materially differ from one another, all being rich in feldspar and showing many accessory

species. It is of interest to note that the feldspar is essentially microcline. The silts are thus characterized by the absence of altered minerals.

Remarks on the mineralogical examination

From the examination of the surface sand fractions, it is seen that the finer the fractions the richer it becomes in feldspar and in the variety of accessory species, which gradually decrease in amounts as the fractions become coarser, because owing to perfect cleavage they are more easily broken into fragments and are consequently less resistant to mechanical forces than quartz. The major portion of the soil is composed of these two minerals.

No apatite was found in the fraction examined. Obviously, therefore, the phosphoric acid in this soil must occur accidentally in other minerals, or has escaped detection as apatite in the clay fractions.

Although zirconium was found by chemical analysis, zircon was not found in the mineralogical examination. However, Professor Honess believes that zircon exists in this soil, but that its identity is masked through admixture with other species. Moreover, it may occur in the amphiboles and pyroxenes which are the same chemically, but differ in their optical properties. They have the general formula $RSiO_3$ in which R may be calcium, manganese, magnesium, zinc, sodium, lithium, aluminum, zirconium or titanium.

Rutile occurred usually in small needles or twinned forms and in some cases enclosed in quartz.

Tourmaline was found as an accessory in the silts as well as the sands, but in very small quantities. Chemical examination, however, shows that a trace of boron is present.

There was considerable limonite in the surface fractions. Professor Honess estimates that about two-thirds of the accessory species in the very fine sand fraction of the surface soil is limonite, which according to Clarke (11, p. 532) may contain all sorts of admixtures of other substances such as calcium, manganese, potassium, aluminum, sodium, phosphorus.

The mica is present as sericite which is a secondary muscovite.

It is interesting to note that orthoclase, if present as such, must be in very small amounts and highly kaolinized. No fresh unaltered samples were to be seen in any of the fractions. The potash feldspars yield usually potassium carbonate on decomposition. On this matter, Professor Honess has written that, "It has been suggested by some that orthoclase and microcline, being identical in chemical composition and so closely related in physical and optical character, are one and the same species, the microcline character of orthoclase being the result of sub-microscopic twinning. This, according to Iddings, produces a mono-symmetric arrangement of the molecules and orthoclase is then characterized by polysymmetry, and consequently not different in crystallization from microcline. But an investigation of these species as found in soils more certainly points to a different conclusion. They are both abundant in the soils of this region, or, more correctly perhaps,

originated here, and, although the microcline has survived erosion and decomposition through the ages, it is practically left untouched as an erosion fragment, although occasionally fragments appear slightly kaolinized. The orthoclase fragments have long since been destroyed, and its presence now is only indicated by occasional kaolinized skeletal cleavage fragments or its alternative equivalent."

The feldspars, according to Professor Honess, are mixed feldspars of acid character, or albite variety, and may contain sodium, calcium or potash.

Rutile, zircon, tourmaline apparently have little or no plant food value.

OCCURRENCE OF TITANIUM, MANGANESE, AND THE RARER ELEMENTS IN PLANTS

The rarer elements are more widely distributed in plants than is commonly supposed which shows that soil conditions are favorable for their accumulation. Inasmuch, moreover, as these elements are present as relatively insoluble compounds, the question arises as to why and how such relatively large amounts become absorbed and assimilated by plants and as to how these elements affect plants.

Titanium. According to Czapek (18) Aderholdt found titanium in plants as early as 1852, Wait (74) found it in oak, apple and pear woods, apples, cow peas and cotton seed meal; and Geilman (35) in a number of plants including apples in amount equal to 0.0004 per cent of the air-dried material. Geilman found that it accumulated by preference in the assimilating organs, which appears to confirm Mosea's work (56) that titanic acid is of great physiological importance to plants because it acts as an oxidizing catalyst.

Barium. According to Watts (76, v. 1, p. 500), Scheele was the first to mention the occurrence of barium in 1788 in beech trees. It was found by Hornberger (43) also in beech trees. In 1875 Dworzak (26) found 0.089 per cent and 0.26 per cent of barium oxide in the stalk and ear of wheat respectively, that had grown in the Nile Valley. Marsh, Alsberg and Black (51) give the results of a number of determinations of barium in alfalfa, barley, millet and beets collected from various parts of Colorado, Wyoming and Arizona. McHargue (52) discussing the occurrence of barium in the tobacco plant and the sycamore tree, concludes that it is present in combination with organic acids and that since it is contained in the live cells of the higher plants it may function in metabolism. Artis and Maxwell (1) report amounts of barium in the leaves of tobacco, dogwood, cottonwood, cherry leaf, black locust, mulberry, elm, maple, plum, walnut, pear, linden, and box-elder varying from 0.005 per cent BaO in the cottonwood to 1.07 per cent in the mulberry. Headden (39, p. 10-17) found it in alfalfa, corn leaves, pea vines and tobacco grown in Colorado in amounts varying from 0.02 to 0.2 per cent BaO. The writer has found 0.22 per cent BaO in alfalfa grown on the Pennsylvania State College Farm.

Caesium. Von Lippman (73) found caesium in the ash of sugar beets.

Rubidium. Pfeiffer (57) reported rubidium in sugar beets. According to Watts (76, v. 5, p. 127), it has also been found in coffee, tea and cocoa.

Vanadium, chromium and molybdenum. Demarcay (24) identified these elements by the spectroscopy in the ash of grapevines, fir and poplar. According to Robinson (62, p. 4), von Lippman found vanadium in the ash of grapevines, sugar beets and various trees.

Lithium. Gaunersdorfer (34) and later Tschermak (68) found lithium in a number of plants and Headden (39, p. 10-17) found it in loco weed, alfalfa and tobacco. The writer has found it in alfalfa grown on the Pennsylvania State College Farm.

Strontium. Headden (39, p. 10-17) found notable amounts in loco weeds, corn leaves and alfalfa. The writer has found 0.10 per cent SrO in alfalfa grown on the Pennsylvania State College Farm.

Boron. Jay (45) found small amounts of boric acid in onions.

Zirconium. The author has not been able to find any mention in the literature of the occurrence of zirconium in plants.

Special mention must be made of the work of Robinson (63, p. 9-17), who has carried out extensive investigations, by methods of unquestionable validity, on the relation of some of the rarer elements in soils and plants. Plants grown on soils in which the rarer elements had been found were examined with the following results:

In apples he found barium, strontium, lithium, rubidium and a trace of titanium; in beets, chromium, vanadium, barium, strontium, lithium, rubidium and caesium; in alfalfa, barium, strontium, lithium and rubidium; in beans, lithium and rubidium; in cabbage and bluegrass, a trace of chromium; in corn, timothy and wheat, titanium, rubidium, strontium, lithium and barium; and in the case of timothy, chromium and caesium.

He concludes that lithium is present in traces in all plants examined and that rubidium is present in the majority of cases in amounts ranging from a trace to 0.005 per cent of the dried material, which is larger than the amounts present of the other rare alkalies. Caesium was found in three plants. Chromium was found in amounts ranging from a trace to 0.004 per cent of the material. Vanadium was found in traces in six plants. Barium was found in all cases and strontium in all samples except bean seed. Molybdenum was not found in any samples.

It is of interest to note in connection with Robinson's work that where the plant contains a comparatively large amount of the rare alkalies there is also an abnormal amount of manganese present.

THE EFFECT OF THE RARER ELEMENTS ON PLANTS

Very little experimental work has been carried out on the physiological action of the rarer elements on plant tissues.

That lithium has a toxic action is shown by the work of Gaunersdorfer (34), Voelcker (71, 72), Frerking (31) and Ravenna and Zamorani (60). Voelcker found that the chloride and iodide applied at the rate of 2 cwt. per acre tends to check root growth in the case of barley, wheat and corn; in some cases actually destroying a proportion of the plants grown; but they found that the application of the oxide at the same rate had a beneficial effect on wheat and barley. Frerking found the poisonous influence of lithium more pronounced than of magnesium in organisms requiring calcium, but not to calcium-free algae and fungi. Very little work has been done on the effect of caesium; but the work of Lucanus (50) and Voelker showed that its salts are less harmful than the salts of lithium.

McHargue (53) states that, although it has been known for more than a century that plants are able to extract appreciable amounts of relatively insoluble compounds of barium contained in soils, little has been done to determine whether or not the compounds of this element have any specific function in vegetable economy. He showed that barium compounds in the absence of calcium carbonate are poisonous to plants; but barium carbonate in the presence of calcium carbonate apparently exerts a stimulating influence.

Haselhoff (38) found that strontium had no injurious action, is absorbed by plants, and the results indicate that it replaces calcium when there is an insufficient supply of that ele-

ment. McHargue's (53) results, however, show that SrCO_3 cannot be substituted for CaCO_3 . In Loew's (49) experiments on algae, strontium salts injuriously affected the chlorophyll bodies and caused loss of starch-making power and finally death. The physiological effect of rubidium chloride has also been investigated by Loew (48), who in pot culture experiments with Chinese barley, cabbage and spinach found that there was a stimulating action in amounts not exceeding 10 mgm. per 50 kgm. of soil; but when applied at the rate of 50 mgm. per 50 kgm. of soil the stimulating effect was not so great except in the case of cabbage as when small quantities were used.

Hebert (40) tested the effect of the sulfates of chromium, aluminum, magnesium, zirconium, thorium, cerium, lanthanum, etc., on germinated seeds of peas, wheat, rape and the yeasts. The toxicity of the different compounds varied slightly on the different groups, but in general occurred in the following diminishing order: Zirconium, thorium, aluminum, chromium, cerium and magnesium. With regard to vanadium, Ramirez (cited by Robinson, 63, p. 9-17) found that it is absorbed and stored by some plants and causes anomalies in their growth. A review of the literature of the presence and effect of boron in plants is given by Cook (12) and Cook and Wilson (13, 14). It would appear that numerous factors influence the absorption, distribution and action of boron on plants. There is evidence to show that below a certain dilution boron tends to produce stronger roots and shoots. This stimulating action appears to be due to the increase of nitrates. Large amounts are known to be toxic to all plants with the exception of certain fungi.

A fairly complete review of the effect of manganese is given by Dietrick (25).

Summarily it would appear that each ash constituent must be considered as exerting some slight influence on the plant. Though plants may develop normally in a nutrient solution without a given element, it does not necessarily follow that this element, if present, might not exert some influence, for the absence or presence of any particular element might be the deciding factor in determining whether or not the plant reaches full development.

Elements like titanium, manganese, barium, boron, strontium, rubidium, etc. may act as catalyzers.

SUMMARY

This introductory paper gives an account of the preliminary work carried out on the soil used in an intensive apple fertilizer experiment conducted in steel rims as an Adams Fund Project. At the present time it is desired to draw attention to the following main facts.

1. The rarer elements vanadium, chromium, molybdenum, zirconium, rubidium, caesium and lithium have been isolated and identified by qualitative and quantitative methods of indisputable validity from a residual soil remote from metamorphic rocks.

2. Positive proof is presented of the presence of barium and strontium, which is the first announcement made of the occurrence of these elements in this phase of the Hagerstown series of soils. The results for BaO are believed to be accurate. However, as will be seen, the results for SrO are approximate. For complete separation from calcium was not obtained.

3. The calcium and magnesium are derived not only from dolomite, but a considerable portion of the quantity of these elements present are contributed by the silicates amphibole and chlorite respectively. Though this

soil is formed from limestone rocks it contains no CaCO_3 . Moreover, magnesium is present in larger amounts than calcium.

4. The chief source of potassium in this soil are microcline and the mixed feldspars of acid variety, on the availability of which contradictory evidence has been obtained by different experimenters. Mica is present only as an accessory. The orthoclase has practically all been kaolinized.

5. The existence of iron in the ferrous condition, notwithstanding difficulties in determining the absolute amounts, has been definitely established.

6. The greater portion of the sulfur exists either in the free state or, as seems most probable, in organic combination.

7. All three soil layers contain only a trace of chlorine (chlorides) which is most certainly remarkable for it is almost invariably present in soils to the extent of about .01 per cent.

8. Accurate methods for the determination of boron and fluorine in soils need to be developed.

9. A review of the literature of the occurrence of the rarer elements in plants and of the experimental work on the effect of some of them is given. The question is raised—how do such relatively large amounts of the rarer elements become absorbed and assimilated by plants, and what function, if any, do they possess?

As this experiment develops it is planned to investigate the absorption not only of the main plant food elements, but also of some of these rarer elements and the author hopes that the presentation of the facts in this paper will stimulate work along similar lines by others. It is fully realized that a new set of variables is presented in our already complex soil problems; but it is only by a correlation of all variables that an advance will be made in our knowledge of these formidable biological problems.

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EFFECT OF CHEMICAL AGENTS ON OXIDATION IN SOIL-FORMING ROCKS AND MINERALS

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INTRODUCTION

In an investigation on the rate and extent of solubility of soil-forming rocks and minerals it was observed that certain rocks and minerals when treated with certain chemical agents and allowed to stand, developed different colors in these various chemical agents. This color development appeared to be due mainly to oxidation or lack of oxidation of the iron present, and is greatly accelerated by some chemical agents and entirely prevented by others. The results appear to throw much light on oxidation and color in soils and in the earth's crust.

METHOD AND PROCEDURE

The material studied was banded granite, biotite granite, ferruginous sandstone, hornblende, mica-schist, pegmatite, quartzite, scotch granite, shale, siliceous sandstone, syenite, apatite, amphibole, apophyllite, crysolite, epidote, kaolinite, limonite, oligoclase, orthoclase, prochlorite, pyroxene, quartz, serpentine, siderite, and stilbite. The chemical agents used were $\text{Ca}(\text{NO}_3)_2$, KNO_3 , KCl , NaCl , $(\text{NH}_4)_2\text{SO}_4$, $\text{NaC}_2\text{H}_3\text{O}_2$, $\text{Ca}(\text{OH})_2$, KOH , NaOH , HCl , HNO_3 , H_2SO_4 , citric acid and oxalic acid. Each rock or mineral was treated by all of these chemical agents. With the exception of $\text{Ca}(\text{OH})_2$ the strength of all these solutions was one-tenth normal.

The rocks and minerals were ground very fine either in an iron mortar or in a grinding machine run by power. By grinding these materials in iron containers they were, of course, contaminated with iron, and it was undoubtedly this free iron which was oxidized or not oxidized under the influence of the different chemical agents, that gave rise to the different colors.

Twenty grams of powdered material and 25 cc. of solution were mixed in a glass tube stoppered with a rubber stopper and allowed to stand for a certain length of time before its solubility was determined. It will be noted that the solid material was submerged in a column of liquid, and that air was excluded from coming in contact with the mixture. It was under these conditions that the color development was first observed. In studying color development more directly and in detail the same general method and procedure were followed,

with few modifications. One of these modifications consisted of adding about 40 cc. of liquid instead of 25 cc. to 20 gms. of solid material and paraffining the mouth of the tube to make absolutely sure that no air entered the tube.

EXPERIMENTAL RESULTS

A new or different color developed in most of the rocks and minerals mentioned above. But the thing that it is most desired to report is the fact that the various chemical agents had distinctly different effects upon this color development within any one rock or mineral. Figure 1 indicates the different colors

Bluish grey	Water	Lead grey	Tan	NaCl
White	$\text{CaH}_4(\text{PO}_4)_2$	Grey	Light tan	$\text{Na}_2\text{C}_2\text{H}_3\text{O}_2$
Brownish yellow	$(\text{NH}_4)_2\text{SO}_4$	Light tan	HNO_3	
Greenish grey	KH_2PO_4	Light grey	H_2SO_4	
Tan	$\text{Ca}(\text{NO}_3)_2$	Greenish grey	HCl	
Greenish grey	Light tan	KNO_3	Whitish grey	Oxalic acid
Lead grey	Tan	KCl	Light grey	$\text{Ca}(\text{OH})_2$

FIG. 1. DEVELOPMENT OF DIFFERENT COLORS IN ORTHOCLASE POWDER TREATED WITH VARIOUS CHEMICAL AGENTS

that developed and the manner of their development, in the case of orthoclase treated with the various chemical agents. This is a typical case of a large number of rocks and minerals tested, except that the intensity or shade of the new color varied according to the native or original color. The orthoclase stood in contact with the respective liquids for over a year.

The color developed in the orthoclase varied considerably with the different chemical agents as well as the rate at which this color developed. Beginning with water, the color was bluish-grey throughout the column; with $\text{CaH}_4(\text{PO}_4)_2$, white throughout; $(\text{NH}_4)_2\text{SO}_4$, brownish yellow throughout; KH_2PO_4 , greenish grey throughout; $\text{Ca}(\text{NO}_3)_2$, tan throughout; KNO_3 , light tan, upper three-

fourths of the column, and greenish grey the lower fourth of the column; KCl, tan upper one-third, and lead grey lower two-thirds; NaCl, tan upper one-third and lead grey lower two-thirds; $\text{NaC}_2\text{H}_3\text{O}_2$, light tan upper one-third and grey lower two-thirds; HNO_3 , light tan throughout; H_2SO_4 , light grey throughout; HCl, greenish grey throughout; oxalic acid, whitish grey throughout; $\text{Ca}(\text{OH})_2$ light grey throughout.

Although the above tendency of color development is typical of a large number of rocks and minerals used, yet in some of the rocks and minerals the shade of the colors varied considerably. This would be naturally expected on account of the difference in the native or original color. The most important thing to bear in mind, however, is that the chemical agents influence this color development and that each chemical agent is usually regular and consistent in producing the same kind of result in all of the rocks and minerals.

Some of these reagents not only help the oxidation to start but even accelerate its rate greatly, while others prevent its inception entirely. $\text{Ca}(\text{NO}_3)_2$ is the best example for the first group and $\text{CaH}_4(\text{PO}_4)_2$ for the second. The $\text{Ca}(\text{NO}_3)_2$ caused a pronounced oxidation in nearly every rock and mineral to which it was added, and at a comparatively rapid rate. In the case of the orthoclase, for instance, the whole column of the material shown in the preceding diagram, was oxidized in about three months, while in such salts as KCl and NaCl it took over fifteen months for only one-third of the column to be oxidized. The $\text{CaH}_4(\text{PO}_4)_2$ prevented oxidation in almost every rock and mineral to which it was added.

The $\text{CaH}_4(\text{PO}_4)_2$ not only prevented oxidation in the various rocks and minerals but it even destroyed the oxidizing influence of the various chemical agents, when it was mixed with them. When the rocks and minerals were treated with any chemical agent containing $\text{CaH}_4(\text{PO}_4)_2$ usually no oxidation appeared. Apparently, the non-oxidizing effect of the $\text{CaH}_4(\text{PO}_4)_2$ is so dominant that it overcomes and masks the oxidizing effect of the other chemical agents.

Oxidation in water alone was not very consistent. In some of the rocks and minerals it developed, while in others it did not. But wherever it did take place, its rate of development was exceedingly slow.

It will be noted from the preceding description and figure 1 that the oxidation started in every case from the top of the column and worked downward, in a very gradual and regular manner. Evidently the oxidation process was obtaining the oxygen from the liquid column which stood above the solid material. It could not obtain it from the outside atmosphere because the tubes were stoppered air tight.

In some experiments the tubes were left open, but the same general type of results were obtained as when they were closed, except that when they were open the rate of oxidation was slightly greater.

As stated previously the rocks and minerals were ground in an iron mortar and it was the free iron which they acquired during the grinding that was oxi-

dized and gave rise to the tan and yellow colors. While no extensive investigation was undertaken to determine what was exactly the form of this oxidized iron, preliminary examination indicated that it was hydrated ferric oxide. In those chemical agents in which oxidation was prevented, the iron probably remained in the free or ferrous state.

Just how the various chemical agents influence oxidation so differently is not definitely known. The oxidation could not be due to the oxygen in the molecule of the reagents because some of them, such as KCl and NaCl, have no oxygen in their molecule and yet cause oxidation, while others that have oxygen in their molecule such as $\text{CaH}_4(\text{PO}_4)_2$, usually cause no oxidation.

The explanation that seems most plausible and satisfactory is to assume that the oxidation in question is caused mainly by catalytic effects, and that the various chemical agents possess different catalytic powers toward oxidation, and consequently affect this process differently. $\text{Ca}(\text{NO}_3)_2$ and $(\text{NH}_4)_2\text{SO}_4$ which induce the most rapid and extensive oxidation may be considered as possessing the most catalytic oxidizing power, $\text{CaH}_4(\text{PO}_4)_2$, KH_2PO_4 , CaCO_3 , $\text{Ca}(\text{OH})_2$ which induce very little or no oxidation may be regarded as possessing very little or no catalytic oxidizing power, while KCl, NaCl, $\text{NaC}_2\text{H}_3\text{O}_2$ etc. may be classed as intermediate. The manner and character of the oxidation in the various chemical agents seem to strongly support this explanation. On the other hand, it is possible that the effect of the different chemical agents upon the hydrolysis or solubility of the iron may also be responsible for the different manner and rate of oxidation.

DISCUSSION

The results reported herein contain at least three important and fundamental facts.

1. The different chemical agents have a very decidedly different effect on the oxidation of iron, both as to rate and extent.
2. The dominant non-oxidizing effect of some of the chemical agents may prevent or mark the oxidizing effect of other chemical agents.
3. The oxidation will take place even when the material is immersed under a deep column of liquid, and even when the freer circulation of atmospheric oxygen is excluded.

These results should tend to throw much light (1) on the oxidation and color of soil at various depths; (2) on the influence of fertilizers on oxidation and color of soils; and (3) on the oxidation and color of the substratum of the earth.

EFFECT OF REACTION ON GROWTH, NODULE FORMATION, AND CALCIUM CONTENT OF ALFALFA, ALSIKE CLOVER AND RED CLOVER¹

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In a former investigation (3) it was found that the reaction most favorable for the growth and nodule formation of soybeans and cowpeas is pH 6 to 7, and that corn tolerates a greater acidity than soybeans and cowpeas. In this paper a report is given on the effect of reaction on the growth, nodule formation and calcium content of alfalfa, alsike clover and red clover.

Since a fairly extensive review of the literature is given in a former paper (3) only a few of the more important papers are here reviewed.

The investigations of Salter and McIlvane (17), Hoagland (10), Duggar (6), Joffe (11), and the writer show that many plants require a slightly acid reaction for maximum growth. Under field conditions, however, alfalfa seems to do best on soils which are approximately neutral or which have some calcium in the form of carbonate.

The increase of nitrogen in many legumes from the addition of lime to acid soils has been noted by Lipman and Blair (12, 13), Morse (15) and others. These results were attributed in part to the favorable influence of the lime on the legume bacteria. No doubt this is in part correct, since the investigations of Fred and Davenport (7) and Bewley and Hutchinson (2) show that most species of legume bacteria grow best at a neutral or slightly alkaline reaction.

The influence of reaction on the assimilation of calcium by plants has recently received considerable attention. Morse (15) found that the addition of lime to acid soils increased the percentage of nitrogen in clover, but decreased the percentage of calcium. Shedd (18) noted that the addition of calcium to six different acid soils either as nitrate, oxalate or citrate produced an increase in the calcium content of many plants. He concluded that the soils were not supplying sufficient calcium for normal plant growth. This agrees with the work of Tokenchi (20) who reported that the addition of lime to the soil increased the percentage of calcium in the oat plants grown thereon.

McIntire (14) states that lime-loving plants grow poorly on acid soils, not because of the acid conditions, but because the soil solution is not sufficiently concentrated in calcium carbonate. He concludes that the reaction of the soil is rarely detrimental to higher plants, though it may be to bacteria and fungi. On the contrary, Olsen (16) and Wherry (23)

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report that there is a correlation between the acidity of the soil and the type of plants grown thereon, which would indicate that the reaction of the soil does have a direct influence on plant growth. The investigations of Salter and McIlvane (17), Duggar (6), Hoagland (10), Conner and Sears (4) and others show that controlled reactions equal to those found in acid soils affect plant growth in the same order as those of the soil.

The investigations of Hartwell and Pember (9), Abbott, Conner and Smalley (1), Conner and Sears (4) and others indicate that some of the unfavorable influences attributed to acid soils are due to soluble aluminum salts. Stoklasa (19), however, noted that small amounts of aluminum salts are beneficial to plants, while large amounts are toxic. He reports that the plant ash always contains aluminium. Denison (5) found only insignificant quantities of soluble aluminum in the water leachings of the acid soils with which he worked. He concluded that any soluble aluminum present in the soil solution is due to the solvent action of the soil acids.

The results of the various investigators indicate that many of the cultivated plants grow best at a slightly acid reaction, while most species of the legume bacteria grow best at a neutral or a slightly alkaline reaction. The reaction of the soil seems to have a considerable influence on the assimilation of calcium by plants and also on the type of plants which grow thereon.

EXPERIMENTAL

The plants were grown in quartz cultures in the greenhouse. For containers, 600-cc. percolators were used. The methods and nutrient solution were the same as those described in a previous paper (3). But a somewhat different procedure was followed in adjusting the reactions. This was done by adding a buffer consisting of $\frac{1}{2}$ gm. of disodium phosphate to a liter of nutrient solution and then adding varying amounts of diluted sulfuric acid or sodium carbonate to portions of the solution to obtain the different reactions of approximately, pH 3, 4, 5, 6, 7, 8, 9 and 10. Sand cultures were used rather than solution cultures, because they furnish a more natural media and are easier to handle, especially for very small seedlings. After the reactions of the sand had become constant by repeated washings with solutions of the desired hydrogen-ion concentration, the seeds were planted and the plants subjected to the different reactions from the time of germination. The sand was inoculated with pure cultures of the proper legume bacteria for two consecutive days as the solutions were renewed. Two weeks after germination the plants were thinned to 6 per culture. When the plants were four months old the sand was washed from the percolators, and notes were made of the growth and the nodule formation.

To determine the amount of calcium in the plant tissue, it was dried, ground to a fine powder, weighed and ignited. The ash was treated with dilute hydrochloric acid. The iron and phosphorus were separated from the calcium by the basic acetate method: a few drops of .05 N ferric chloride were added to make sure that all the phosphorus would be precipitated in the form of ferric phosphate. The calcium was precipitated as the oxalate, and determined by titration with standard potassium permanganate.

It was noted that the different reactions pH 3 to pH 10 had no appreciable harmful effects on the germination of any of the seeds, but in all cases the seedlings began to die immediately in the most acid and alkaline cultures. Duplicates agreed throughout. The diurnal changes in reaction of the nutrient solution due to the plants were in proportion to the plant growth. However, at optimum reactions no changes occurred. The behavior of the different plants in changing the reaction of the nutrient solution was practically identical; this change varied from nothing to 0.7 pH.

All the alfalfa seedlings died at pH 3, and only one lived at pH 4, while 5 lived at pH 9, and 4 at pH 5. All the seedlings lived at pH 7 and 8. A very good idea of the growth and size of the plants may be obtained from plate 1. The plants at pH 4, 5 and 6 were somewhat yellow during the entire experiment; those at pH 9 and 10 were green but had few branches. Plants at pH 7 and 8 were decidedly the best. Nodules formed at all reactions at which the plants grew, but they were most profuse at pH 6, 7 and 8. An increase in acidity and alkalinity produced a decrease in plant growth and nodule formation. A decrease in acidity produced an increase in the percentage of calcium in the plant tissue. The comparative growth and the percentage of calcium in the plants at pH 5, 7 and 9 are given in table 1.

At pH 3 and 4 all the alsike clover seedlings died, while only one died at pH 10, and none at any other reaction. The plants which lived grew vigorously at all reactions, except those at pH 9 and pH 10, which were yellow and had slightly brown roots. Nodules formed at all reactions as may be seen from plate 2 which gives the comparative growth of the plants at the different reactions. The best plant growth took place at pH 7 and pH 8; the growth at pH 8 was a trifle better than that at pH 7. The acid reactions, pH 5 and pH 6, showed no injurious effects on the roots of plants though the growth was less at these reactions than at pH 7 and pH 8. The percentage of calcium in these plants increased as the acidity decreased. Table 2 gives the results with the alsike clover grown at the different reactions.

At pH 3 and pH 10 all the red clover seedlings died; an average of three lived at pH 4, five at pH 5 and three at pH 9. Because of an infestation of red spider on the clover, it did not produce so vigorous a growth as did the other two legumes. The comparative growth of this clover at the different reactions is given in plate 3. It will be seen that the roots of these plants are slightly darker than those of the other two series. The dark appearance is due in part to light conditions when the photograph was taken. The roots at pH 4 and pH 10 show a lighter color than those at pH 6 and pH 7. In reality the color was the reverse. The best plant growth was noted at pH 7 and pH 8. Wherever the plants grew, nodules formed. An increase in acidity and alkalinity produced a decrease in plant growth and nodule formation. A decrease in acidity produced an increase in the percentage of calcium in the plant. Table 3 gives the comparative plant growth at the different reactions, and the percentage of calcium in the plants at pH 5, pH 7 and pH 9.

TABLE 1

The growth, nodule formation, and percentage of calcium of alfalfa at different reactions

REACTION	NUMBER OF PLANTS		GROWTH	NODULES	DRY WEIGHT, AVERAGE OF 10 PLANTS	CaO
	Initial	Final				
pH					gm.	per cent
3	6	0	Dead	0
4	6	1.0	Yellow	Poor
5	6	3.5	Yellow	Fair	1.92	1.01
6	6	5.5	Fair growth	Good	2.26
7	6	6.0	Good	Good	4.10	1.23
8	6	6.0	Good	Good	4.00
9	6	4.5	Green but small	Good	2.70	1.36
10	6	1.0	Green but small	Fair	2.20

TABLE 2

The growth, nodule formation and percentage of calcium of alsike clover at different reactions

REACTION	NUMBER OF PLANTS		GROWTH	NODULES	DRY WEIGHT, AVERAGE OF 10 PLANTS	CaO
	Initial	Final				
pH					gm.	per cent
3	6	0	Dead
4	6	0	Dead
5	6	6	Fair growth	Good	1.31	0.97
6	6	6	Good	Good	2.20
7	6	6	Good	Good	2.55	1.13
8	6	6	Good	Good	2.90
9	6	6	Yellow	Good	1.30	1.38
10	6	6	Yellow	Fair	1.07

TABLE 3

The growth, nodule formation and percentage of calcium of red clover at different reactions

REACTION	NUMBER OF PLANTS		GROWTH	NODULES	DRY WEIGHT, AVERAGE OF 10 PLANTS	CaO
	Initial	Final				
pH					gm.	per cent
3	6	0	Dead
4	6	3	Poor	Poor	0.8
5	6	5	Fair	Fair	1.64	1.02
6	6	6	Good	Good	2.40
7	6	6	Good	Good	2.91	1.07
8	6	6	Good	Good	3.01
9	6	3	Fair	Poor	1.20	1.42
10	6	0	Dead

DISCUSSION

Strong acidity and alkalinity would not be expected to hinder germination of seeds as much as the growth of the seedlings. The very young seedlings are more sensitive to unfavorable reactions, because of the small quantities of buffer material present. In general, the power of the seedling to withstand unfavorable conditions is in proportion to the size and age of seedling. It is possible for large seeds to supply nourishment to the seedlings several days, thereby keeping them alive; but when the reaction of the medium is very unfavorable, the seedlings soon die after the nourishment in the cotyledons is utilized.

The alfalfa and clover bacteria functioned in nodule formation at a more alkaline reaction than did the soybean and cowpea bacteria, while the limits of nodule formation in the acid range were about the same for all the plants. The results thus far indicate that nodules form wherever root hairs are produced. The formation of only a few nodules on the roots of soybeans and cowpeas outside of the range pH 4 to pH 8 is believed to be due to the absence of root hairs. It is possible for plants to produce a meager growth in solution cultures without the formation of root hairs.

Field observations show that alfalfa responds to lime much more than do soybeans; and the work of Fred and Davenport (7) show that the alfalfa bacteria are less resistant to acidity in pure cultures than the soybean bacteria. But it would be unfair to say that the difference in response of these plants to lime is due more to the influence of the reaction on the respective bacteria than on the plants themselves.

The cause of the difference in effects of the various reactions on the growth of alfalfa and clover is not readily explained because of the number of factors involved. Possibly the most plausible explanation thus far is to be found in the difference in reaction of the cell sap of these plants under normal conditions. The results of Haas (8) and Truog and Meacham (21) show that the reaction of the cell sap of both red and alsike clover is more acid than that of alfalfa. The figures will show clearly that both clovers grew at acid reactions, pH 5 and pH 6, better than did alfalfa, even though one alfalfa seedling survived at pH 4. The results seem to be in keeping with Truog's (22) suggestion concerning the feeding power of plants in an acid medium.

Assuming that most of the calcium in plants acts as a neutralizing and precipitating agent of the by-product in the plant sap from the vital processes, it appears that plants grown at different reactions but with equal amounts of calcium in solution, would have a greater percentage of calcium when grown in the more alkaline range as long as fair plant growth took place, because the acid conditions hinder the precipitation of the calcium salts. Tables 1, 2 and 3 show this to be true. The reaction of the media at which the plants are grown seems to influence the power of the plants to obtain sufficient calcium for normal growth. Since the roots of the plants which sur-

vived the acid reactions, pH 5 and pH 6, showed little or no toxic effects from the acidity, it seems that, due to the acidity, the plants were not getting sufficient nutritives for normal growth. These results show that the reaction of the media in which the plants are grown influences the power of the plant to secure sufficient calcium for normal growth even though the quantities of soluble calcium present are approximately equal. This does not appear to be in accord with McIntire's (14) suggestion that the reaction of the soil is rarely injurious to higher plants.

The influence of reaction may bring about a similar change with other nutritive elements; the effect on nitrogen has already been noted. The results reported with calcium are an average of four determinations.

The very alkaline condition possibly precipitated the iron so completely that the clovers were unable to obtain sufficient amounts of iron for normal growth, and hence, produced a chlorotic effect; this was not apparent, however, with the alfalfa.

The results show distinctly that the reaction of the media in which the plants were grown has a direct influence on the growth and nodule formation of the plants. The reactions which were injurious to growth and nodule formation were within the range of reactions of actual soil solutions and suspensions. Thus the necessity for the proper adjustment of the soil reaction for maximum growth and nodule formation is apparent. Acidity and alkalinity produce toxic effects directly, and also influence the power of the plant to obtain sufficient calcium for normal growth. Further studies on the influence of reaction on plant growth and nodule formation under field conditions are in progress.

SUMMARY AND CONCLUSIONS

The effects of different reactions on alfalfa, alsike clover and red clover were studied. These plants were grown in quartz sand cultures using a modified form of Crone's nutrient solution adjusted to the reactions desired. The reactions were kept as constant as possible by the use of two buffers, di-sodium phosphate and sodium carbonate and by renewing the solutions daily. The plants were allowed to grow for four months.

The results may be summarized as follows:

1. Alfalfa and clover seeds will germinate at a reaction which is too acid or too alkaline for the growth of the seedlings.
2. Very young alfalfa and clover seedlings are much more sensitive to acidity and alkalinity than older plants.
3. Red clover seed will germinate and grow to a small extent at pH 4 but has difficulty in becoming established.
4. Alfalfa seedlings will not establish themselves at pH 4, nor will alsike clover; but the alsike clover does better at pH 5 and pH 6 than alfalfa and red clover.

5. Alfalfa and clover produce maximum growth and number of nodules at pH 7 and pH 8, that is, at neutrality or at a slight alkalinity.

6. Alsike and red clover grow better in an acid reaction, pH 5 and pH 6, than alfalfa; alsike clover withstands a very alkaline reaction even better than alfalfa.

7. Nodules form at any reaction at which the plants grow, but the greatest number are formed at or near neutrality.

8. The critical hydrogen-ion concentration differs slightly for the different plants studied; in general it was about pH 4 for all the plants. Red clover has a somewhat higher critical acid concentration than alfalfa and alsike clover.

9. The critical hydroxyl-ion concentration for alfalfa and red clover was about pH 9 to pH 10, while that for alsike clover was somewhat higher.

10. The greater the acidity of the media in which the plants are grown, the less the power of the plants to obtain the basic substance calcium for metabolic processes.

11. The acidity which was found to be injurious to alfalfa, red clover and alsike clover was not any greater and sometimes less than that found in many acid soils.

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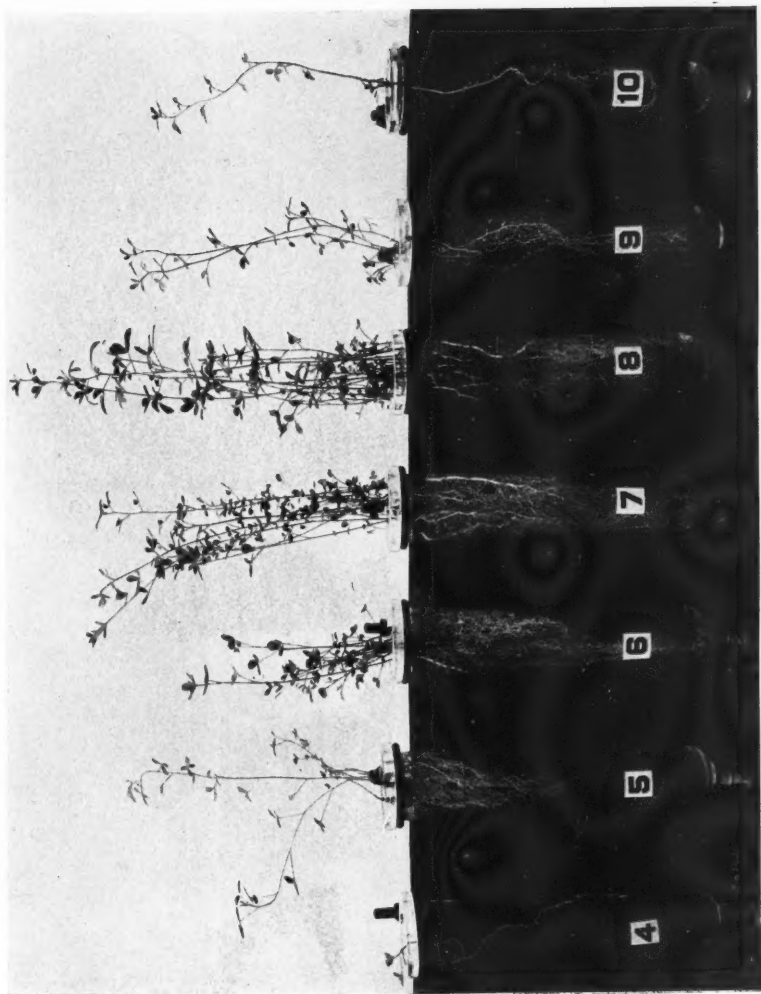
PLATE 1

ALFALFA GROWN IN SAND CULTURES OF DIFFERENT REACTIONS INDICATED BY NUMBERS
WHICH GIVE THE APPROXIMATE pH VALUES

The sand was removed and replaced with water in order to show the root growth and nodule formation.

EFFECT OF REACTION ON SOME LEGUMES
O. C. BRYAN

PLATE I



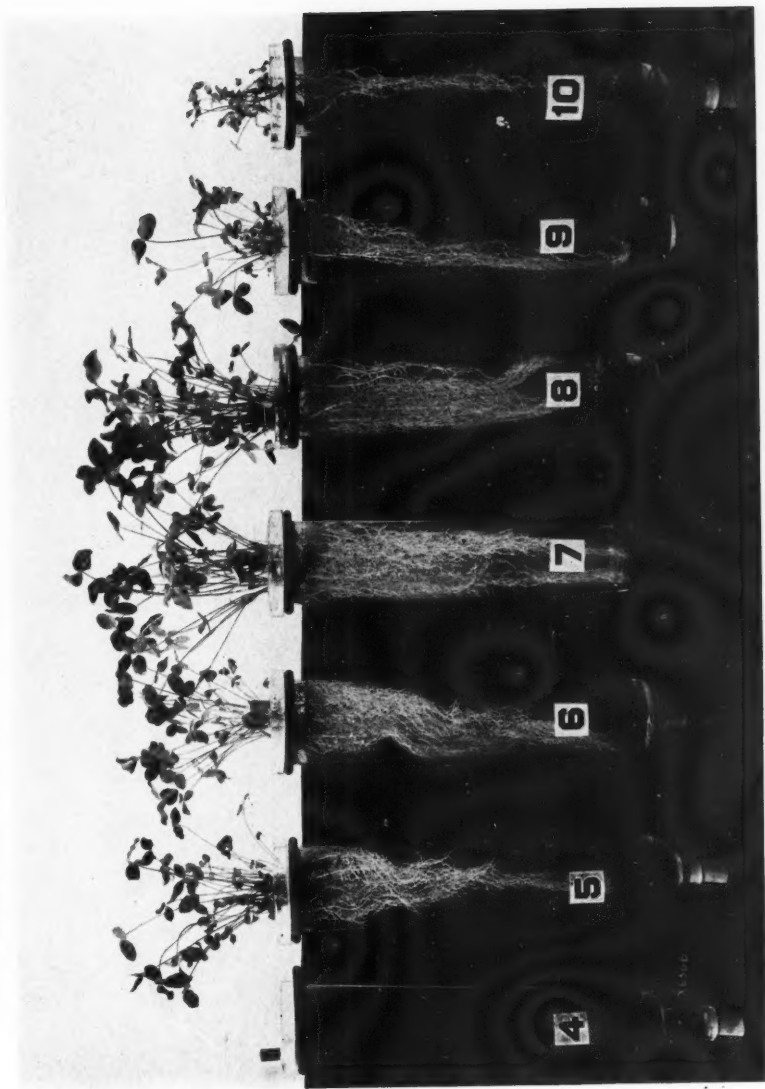
ALFALFA

PLATE 2

ALSIKE CLOVER GROWN IN SAND CULTURES OF DIFFERENT REACTIONS INDICATED BY
NUMBERS WHICH GIVE THE APPROXIMATE pH VALUES

The sand was removed and replaced with water in order to show the root growth and
nodule formation.

EFFECT OF REACTION ON SOME LEGUMES
O. C. IRVING



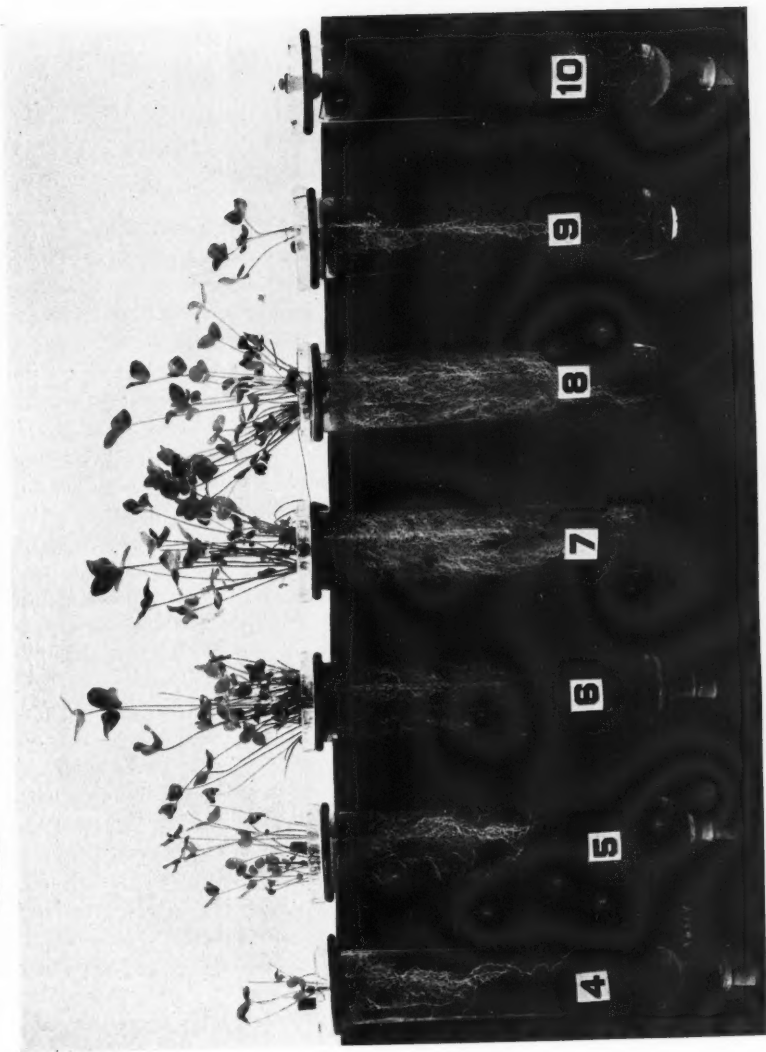
ALSKE

PLATE 3

RED CLOVER GROWN IN SAND CULTURES OF DIFFERENT REACTIONS INDICATED BY NUMBERS
WHICH GIVE THE APPROXIMATE pH VALUES

The sand was removed and replaced with water in order to show the root growth and
nodule formation.

EFFECT OF REACTION ON SOME LEGUMES
O. C. BRYAN



RED CLOVER

EFFECT OF ACID SOILS ON NODULE-FORMING BACTERIA¹

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It is generally recognized that the nodule bacteria are sensitive in varying degrees to acidity and hence some investigators (4) have suggested classifying the different species of nodule bacteria according to their critical hydrogen-ion concentration. This arrangement is based entirely on laboratory studies and does not prove that the legume bacteria will behave similarly in acid soil. Bewley and Hutchinson (1) reported that a number of species of nodule bacteria were either killed or rendered inactive in definitely acid soils, but the degree of acidity was not given. Whether or not these bacteria are killed in acid soils, and at what reaction they cease to produce nodules on the host plants are questions of considerable importance from both a practical and a scientific standpoint. The present investigation was undertaken to study the effect of acid soils of known reactions on a few of the important nodule-producing organisms.

Two-hundred-gram portions of five different acid soils ranging from sand to fine sandy loam and silt loam were treated with varying amounts of lime to obtain a series of different reactions for each soil. Portions of the soil so treated were placed in large cotton-stoppered Pyrex glass tubes and inoculated separately with suspensions of alfalfa, red clover, and soybean bacteria. The tubes were kept at room temperature and optimum moisture was maintained by frequent additions of sterile water.

At the end of 75 days heavy suspensions of each soil representing the different reactions were made in sterilized water, and the suspension from each was poured upon sand cultures of alfalfa, red clover, and soybean seedlings previously treated to free them from legume bacteria. The seedlings were allowed to grow for 5 to 7 weeks during which time sterilized water was added from time to time to keep the moisture favorable for growth. The results of nodule formation on these plants for each kind of soil and for each reaction are given in table 1.

The results presented in the table agree with field observations; namely, that the nodule bacteria are killed in acid soils of a certain degree of acidity

¹ Approved for publication by the Director of the Wisconsin Agricultural Experiment Station.

² The writer wishes to express his appreciation for the helpful suggestions and criticisms tendered by Professors E. B. Fred and E. Truog.

TABLE 1

The ability of alfalfa, red clover and soybean bacteria to produce nodules after 75 days incubation in acid soils

TEXTURE	LIME APPLIED PER ACRE	RE- ACTION	NODULE FORMATION FROM INOCULATION WITH THESE SOILS		
			Alfalfa plants	Red clover plants	Soybean plants
	<i>tons</i>	<i>pH</i>			
Sand.....	0	5.8	*	*	*
	1	6.1	*	*	*
	2	6.5	*	*	*
Fine sandy loam.....	0	4.9	None	*	*
	1	5.3	*	*	*
	2	5.8	*	*	*
Silt loam.....	0	5.4	*	*	*
	1	5.7	*	*	*
	2	6.0	*	*	*
Silt loam.....	0	4.5	None	None	*
	1	5.1	Few nodules	*	*
	2	5.5	*	*	*
Silt loam.....	0	3.4	None	None	None
	1	4.2	None	None	*
	2	5.3	*	*	*

* Asterisk denotes presence of nodules.

TABLE 2

The effect of acid soils on the nodule-producing power of alfalfa bacteria

TEXTURE	TREATMENT	RE- ACTION	NODULES
		<i>pH</i>	
Fine sand.....	Control	4.86	None
	Potassium chloride	4.51	None
Peat.....	Control	4.70	None
	Lime	5.4	Nodules
Silt loam.....	Control	5.36	None
	Sodium phosphate	6.1	Nodules
Sand.....	Lime	5.96	Few nodules
	Control	4.9	None
	Lime	5.3	Nodules

within a few months. The limiting hydrogen-ion concentration for the alfalfa bacteria was at pH 5.0; for the red clover, pH 4.5 to 4.7; for the soybean, pH 3.5 to 3.9 regardless of the type of soil. The critical hydrogen-ion concentration for these bacteria in soils agrees with that for the same nodule bacteria in pure culture as reported by Fred and Davenport (4).

In the present investigation nodules were formed on the soybeans at a greater acidity than they were in solution cultures of soybeans in a previous investigation (2). This difference may be accounted for by the absence of root hairs in the very acid range of the solution cultures. On the other hand, alfalfa and clover failed to form nodules at so high a degree of acidity as was noted in another investigation (3) with quartz cultures of these two plants. Possibly the very large number of bacteria used in inoculating these quartz cultures resulted in some of the bacteria entering the root hairs before they had become injured by the acid. The legume bacteria after they have entered the root hairs of the host plant are either better protected from, or more resistant to the injurious effect of acids in the medium in which the host plant is growing.

Microscopic studies were made of alfalfa, red clover, and soybean bacteria which were grown on agar slants of different hydrogen-ion concentrations. It was found that an acid reaction reduced the normal size and retarded the life cycle of these bacteria.

Since there was a fairly definite limit for nodule formation determined by the reaction of the soil in which the bacteria had been kept, it was thought well to note the effect of several other acid soils on the formation of nodules on alfalfa. Four soils (fine sand, sand, silt loam, and peat) were being tested for fertilizer requirements of alfalfa and had been planted to this crop in 2-gallon jars; suspensions of alfalfa bacteria had been added to each jar. Several of these soils produced only small seedlings, some of which were yellow indicating that the plants had not become inoculated. Examinations were made of these plants for nodules, and the reaction of the respective soils was determined electrometrically. The results are given in table 2. The limiting reaction for nodule formation with all the different soils was about pH 5 for the alfalfa bacteria. There was one soil, silt loam, which failed to produce favorable conditions for nodule formation at pH 5.36. The data presented, however, are not sufficient to attribute the absence of alfalfa nodules at pH 5.36 to soil type.

The preceding experiments bring out the need for liming acid soils and frequent inoculation of them. The detrimental action of soil acids on the legume bacteria no doubt accounts for the failure of some crops to become inoculated although the seeds have been treated with the desired bacteria.

SUMMARY

The ability of alfalfa, red clover and soybean bacteria to live and produce nodules, 75 days after being placed in acid soils of different degrees of acidity, was studied with results as follows:

1. The alfalfa bacteria were killed at about pH 5; the red clover bacteria at pH 4.5 to 4.7; and the soybean bacteria at pH 3.5 to 3.9.
2. The critical hydrogen-ion concentration in the soil is approximately the same as in pure cultures.
3. The texture of the soil seems to have no appreciable influence on the critical hydrogen-ion concentration for the bacteria studied.
4. The necessity of frequent inoculation and liming of acid soils is indicated.

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ACID PHOSPHATE PRODUCTION BY THE LIPMAN PROCESS: II. BUILDING UP SULFUR-FLOATS-SOIL MIXTURES WITH A HIGH CONTENT OF TOTAL AND SOLUBLE PHOSPHATE¹

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The slowing down of the conversion of insoluble phosphates with increase in bulk of the floats in the sulfur-floats-soil mixtures and the speeding up of conversion with diluting the mixtures, suggested the idea of gradual additions of floats and sulfur in the proper proportions to the dilute mixtures in order to build up the concentrated mixture.

EXPERIMENT 2: INITIAL STAGE

The following mixture was made up: 75 gm. greenhouse soil, 15 gm. floats and 10 gm. of sulfur. Of this mixture, 800 gm. was placed in each of 2 pots, the layer being 4-5 inch deep. The moisture-content was kept at 50 per cent of the total moisture-holding capacity until the hydrogen-ion concentration went down to pH 2.8. It was then raised to 60 per cent of the total moisture-holding capacity. Experiment 1 described in the first paper of this series suggested such a procedure. The sulfur was increased with a definite idea in mind based on the principles of the mass law. This law tells us, that the velocity of any reaction depends upon the mass of the active ingredients involved and is at any moment proportional to the molecular concentration of the reacting components and a constant which is characteristic of the chemical nature of the reacting substances. The increase of sulfur increased the sphere of activities of the sulfur-oxidizing organisms. The greater mass of sulfuric acid thus formed thus increased the velocity of the reaction involved in the transformation of the insoluble phosphates.

Table 1 gives the course of reaction and transformation of the insoluble phosphates.

The rapid increase of the available phosphates as the hydrogen-ion concentration of the water extract increased, the large amount of sulfur oxidized, and the still greater increase of the hydrogen-ion concentration after all of the insoluble phosphates had been converted into soluble form are significant

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TABLE 1

Course of reaction and transformation of insoluble phosphates in dilute sulfur-floats-soil mixtures

AGE OF CULTURE	CULTURE 1		CULTURE 2	
	Reaction	Available phosphorus per 100 gm. of mixture	Reaction	Available phosphorus per 100 gm. of mixture
weeks	pH	mgm.	pH	mgm.
1	5.0	31.6	5.0	32.7
2	3.4	41.8	3.4	43.9
3	3.0	72.7	3.0	73.4
4	2.8	99.4	2.8	101.3
5	2.8	129.7	2.8	128.9
6	2.6	179.9	2.6	183.6
7*	2.2	186.7	2.2	187.4
8	1.6	183.4	1.6	182.7

* After the seventh week a one per cent HCl extract showed that 6.47 gm. of sulfur had been oxidized.

and instructive phenomena. First, they illustrate the fundamental reactions involved in the formation of soluble phosphates when rock phosphate is treated with sulfuric acid. Second, they hasten the accumulation of phosphoric and sulfuric acids, which is utilized in the later stages of building up the composts. The reactions of transformation of insoluble phosphates to the soluble form belong to the type of reactions of heterogeneous system. In such heterogeneous system the speed of the reaction is a function of a greater number of variables than in the case of a homogeneous system. According to Kazakov (2, 3), there are factors which are common to both systems and they are:

1. Concentration of the reacting mass
2. Temperature of the reacting medium
3. Amount of contact of the reacting substances
4. Speed of diffusion of the reacting substances
5. Catalytic agents

Besides these factors we have others in a heterogeneous system where solid solution phases occur. These are:²

6. Size of contact surface³
7. Chemical composition of the solid phase
8. Physical properties of the solid phase
9. Influence of formation of a solid phase as a result of the reaction

² Some parts of Kazakov's work have been reported already but are treated here more extensively for the sake of completeness.

³ The size of the particles of the rock in the manufacture of acid phosphate has a tremendous influence. Theoretically, all other conditions being equal, the speed of solution of a solid in a liquid is proportional to the contact surface, and in spherical bodies (as we suppose in fine floats) the surface is proportional to the square of the radius. Particles with a radius of 0.1 mm. will dissolve twenty-five times as fast as particles with a 0.5 mm. radius.

Factors 7 and 8 have a tremendous influence on the speed of the reaction and they are the least known, since the chemical make-up of the rock phosphate is still obscure. Kazakov (2, 3) formulates the reactions involved in the formation of soluble phosphates as follows:

TREATMENT	RESULTS	
	Liquid phases	Solid phases
H ₂ SO ₄ added in excess . . .	H ₃ PO ₄ , H ₂ SO ₄ , sulfates of Ca, Al, Fe	CaSO ₄ ·2H ₂ O
Close to optimum	H ₃ PO ₄ , sulfates of Ca, Al, Fe	CaSO ₄ ·2H ₂ O
Optimum	H ₃ PO ₄ , sulfates of Ca, phosphates of Al and Fe	CaSO ₄ ·2H ₂ O
Not enough acid	H ₃ PO ₄ , sulfates of Ca, phosphates of Ca, Al and Fe	CaSO ₄ ·2H ₂ O and part of undissolved phosphate

In the sulfur-floats-soil mixtures the amount of sulfuric acid formed at any moment was small and in the presence of large amounts of tricalcium phosphate there was no accumulation of acid. Besides these purely chemical considerations the biological factor should not to be lost sight of. As yet it is not established at what concentration of the soluble substances formed by the sulfur-oxidizing organisms, become toxic and hinder the activities of the organisms. But leaving this unknown factor aside for the present, let us follow up the scheme of reactions a little further. In the system of sulfur-floats-soil mixtures the liquid phases possible are H₃PO₄, sulfates and phosphates of calcium. In the solid phase we find gypsum and undissolved phosphates. Undoubtedly the liquid phase of the phosphates is in the form of primary phosphates. These react with the tertiary phosphates and bring them in solution. An accumulation of the soluble phosphates lowers the concentration of the soluble CaSO₄·2H₂O, after an initial increase, as shown in figure 1, which is based on Kazakov's data (2, 3). It is very likely that the solubility of the gypsum is the retarding factor in the sulfur-oxidizing activities of the organisms, since high concentrations of phosphates do not seem to effect their activities. This occurs when the mixtures reach a pH value of 2.8-2.6. At this point the transformation of the phosphates gains momentum, phosphoric acid accumulates and a rapid oxidation of sulfur may be noticed. If it were only possible to keep the mixtures under such conditions, adding gradually small amounts of insoluble phosphates the process might be shortened to such a time period that it would be practical on a factory scale.

Tables 1 and 2 illustrate the second phenomenon, namely the accumulation of phosphoric and sulfuric acid which is utilized in the building up of concentrated compost. The material from this experiment was used as a base mixture and will be referred to as "stock culture."

EXPERIMENT 3

From the stock culture of experiment 2 the following mixtures were made up:

CULTURE NUMBER	CULTURE MATERIAL	GREENHOUSE SOIL	FLOATS	SULFUR
	gm.	gm.	gm.	gm.
1	50	25	15	5
2	50	15	25	10
3	50	10	25	15
4	50	0	25	25
5 and 6	150		25	10
7 and 8	150		45	15
9 and 10	150		65.5	22.5
11 and 12	150		25	25

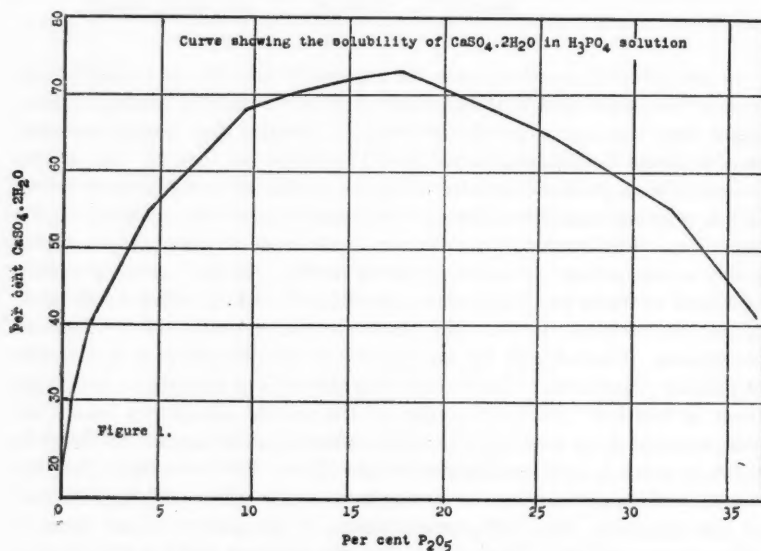


FIG. 1. SOLUBILITY OF $CaSO_4 \cdot 2H_2O$ IN H_3PO_4 SOLUTION

The cultures were placed in tumblers, except 7, 8, 9 and 10 which were kept in small pots. They were incubated at a temperature of 25 to 28°C. Table 2 gives the results of the experiment.

These data show that the inert material, greenhouse soil, is of importance in speeding up the activities of the sulfur oxidizing organisms. Culture 1 may be taken as an illustration. The beneficial effect of organic material on sulfur oxidation is not due to the importance of the nitrogen in the organic matter in the metabolism of the sulfur oxidizing organisms as Rudolfs (5) attempted to show. The organic matter seems to serve as a temporary storage

place for the acid produced by the sulfur-oxidizing organisms, thus removing the sulfuric acid as fast as it is formed. That organic substances have no direct beneficial effect on the activities of the sulfur-oxidizing organisms is pointed out in discussing the effect of organic substances on *Thiobacillus thiooxidans* (1). The data on cultures 1, 2, 3 and 4 illustrate how the organic matter influences the solubility of the phosphates which serve as a measure of the sulfur oxidized. Cultures 2 and 4 contained the same amounts of rock phosphate, and although culture 4 had more sulfur it was behind in solubilizing capacity. The amount of greenhouse soil added to culture 2 is the factor which made it superior to culture 4. An inspection of the data after 7 weeks of

TABLE 2

CULTURE NUMBER	AFTER 1 WEEK			AFTER 4 WEEKS		AFTER 7 WEEKS			AFTER 14 WEEKS		
	Reaction	P in 1 gm. of mixture	Citrate soluble P in 1 gm. of mixture	Reaction	Citrate soluble P in 1 gm. of mixture	Reaction	P in 1 gm. of mixture	Citrate soluble P in 1 gm. of mixture	Reaction	P in 1 gm. of mixture	Citrate soluble P in 1 gm. of mixture
	pH	mgm.	mgm.	pH	mgm.	pH	mgm.	mgm.	pH	mgm.	mgm.
1	3.8	30.4	12.99	2.8	18.76	2.1	27.8	25.96	1.8	27.6	26.4
2	4.4	45.6	10.36	2.8	20.36	2.6	42.0	25.74	2.4	41.7	30.04
3	4.4	44.8	9.56	2.8	19.52	2.6	43.1	23.3	2.4	42.8	29.56
4	4.2	43.2	9.84	3.0	13.88	2.8	41.6	20.68	2.4	40.0	25.2
5	3.0	41.8	14.4	2.8	22.56	2.5	38.2	25.16	1.8	38.6	26.48
6	3.0	41.8		2.8	23.36	2.5	38.2	25.16	1.8	38.8	27.68
7	3.8	44.3	13.36	3.0	16.88	2.6	39.4	24.0	2.4	37.85	29.76
8	3.8	44.3		2.8	20.8	2.4	39.2	25.2	2.2	37.73	30.07
9	3.6	56.8	13.46	3.0	14.64	2.6	51.8	24.0	2.2	51.5	28.8
10	3.6	56.8		2.8	19.52	2.6	51.7	24.0	2.2	51.5	29.56
11	3.2	40.1	14.4	2.4	21.0	2.2	36.2	24.0	1.8	35.7	27.96
12	3.2	40.1		2.4	21.48	2.2	36.2	24.2	1.9	36.1	25.92

incubation shows that in culture 1 practically the entire amount of insoluble phosphates has been converted into soluble forms. But it is important to note that the total amount of phosphorus was only 2.7 per cent, and the bulkiness of such mixtures, from a commercial standpoint, will bar it from practical application. Cultures 11 and 12, because of the large amount of the original culture material, indicate the influence of inoculation material. On the other hand cultures 9 and 10 show that the process has tremendous possibilities. Close to 50 per cent of the total phosphorus was solubilized, notwithstanding the high concentration of total phosphorus. In terms P_2O_5 the total phosphates amounted to 12 per cent of the mixture. It is worth mentioning that after the next 7 weeks of incubation only 60 per cent of the

total phosphates had become soluble in mixtures 9 and 10. Still more prolonged incubation brought into solution more of the insoluble phosphates. Thus within 24 weeks over 75 per cent of the total phosphorus was made soluble; but most of the cultures were not incubated that long. After the tenth week of incubation, the next step of building up the total percentage of phosphates was begun. This step is discussed under experiment 4.

EXPERIMENT 4: SECOND AND THIRD STAGES

Culture 1 received a mixture of 4 parts of rock phosphate to one part of sulfur, whereby the total phosphate content should not have been any greater than 6 per cent. Cultures 2, 3 and 4 were mixed, air dried and after 5.5 weeks a 5 to 1 mixture of rock phosphate and sulfur was added to make the total phosphorus content about 6 per cent; this was called culture A. Cultures 5, 6, 7 and 8 were mixed and a 4 to 1 mixture of rock phosphate and sulfur was added to make a total phosphorous content of not more than 8 per cent; this was called culture B. Cultures 9, 11, and 12 were mixed, air dried, kept for 5 weeks and then rock phosphate was added to this mixture to make a total phosphorous content of not more than 6 per cent; this was called culture C. No sulfur was added because of the excess of sulfur added to these cultures originally.

Table 3 gives the results of this experiment.

An analysis of these results shows outstanding features which should lead to definite conclusions. A few points are, however, interesting. First, the cultures with an original high organic-content showed more efficient sulfur-oxidizing capacity. Culture 1 may again be cited as an illustration. After 3.5 weeks of incubation in the second stage 55 per cent of the phosphates out of a total 5.7 per cent P were made soluble. A new addition of rock and sulfur was made and after another 6 weeks' incubation more than 50 per cent of a total of 6.32 P or 15 per cent P_2O_5 , became soluble. Second, where the concentration of the phosphates becomes higher the second stage is prolonged; culture B is an illustration. The amount of soluble phosphorus approached 56 per cent of a total of 6.98 per cent of phosphorus or 16 per cent P_2O_5 , but the period was prolonged to 20 weeks. Culture C with a slightly lower total of P shows 64 per cent of soluble P in 18 weeks. It is possible that the more rapid activity of this culture was due to the new method of handling the culture. It was air dried to see whether any of the original soluble phosphates would revert upon drying; analyses showed no signs of reversion. The short period of air drying seems to have no effect on the sulfur-oxidizing organisms; if anything it stimulated the activities, a phenomenon well known in soil microbiology. The failure of culture A to show activity is due to the fact that this culture was subjected to intermittent air dryings and moistening.

The investigations of the sulfur-floats-soil mixture were conducted with the aim to reach a mixture with a high total concentration of phosphorus and as much available phosphorus as possible in the shortest period possible. Thus

far mixture 1 was the best, and the results were accomplished in three stages: the first stage 7 weeks, the second 3.5 weeks, the third 8 weeks; the last stage probably could have been shortened 2 weeks, since the addition of the rock phosphate in the third stage could have been made two weeks earlier. Even then the total period for building up mixture 1 was 18.5 weeks, besides the time necessary for the preparation of the original culture. Combining the latter it took 24 weeks to get a mixture of 15 per cent total P_2O_5 with 50 per cent of it soluble. However, if these results are compared with those of McLean (4), Rudolfs (5) and the earlier work of the author they become significant. McLean (4) stated: "In regard to the question as to whether sufficient P_2O_5

TABLE 3

Course of conversion of insoluble phosphates into soluble form in the second and third stages of the Lipman process

INCUBATION PERIOD	CULTURE 1			CULTURE A			CULTURE B			CULTURE C		
	Reaction	P in 1 gm. of mixture	Citrate soluble P in 1 gm. of mixture	Reaction	P in 1 gm. of mixture	Citrate soluble P in 1 gm. of mixture	Reaction	P in 1 gm. of mixture	Citrate soluble P in 1 gm. of mixture	Reaction	P in 1 gm. of mixture	Citrate soluble P in 1 gm. of mixture
weeks	pH	mgm.	mgm.	pH	mgm.	mgm.	pH	mgm.	mgm.	pH	mgm.	mgm.
0.0	2.6	58.48	20.11	3.4	60.1*	17.0	3.6	77.6	20.9	4.0	58.6	23.0
3.5	2.8	57.45	31.93	2.8	73.1	24.8
5.5	3.6†	66.35	15.00	2.8	62.6	21.2
9	2.4	57.4	33.9
11.5	2.8	63.25	31.9	2.3	...	32.45
18	2.4	57.2	36.95
20	2.4	69.8	39.19

* The low figure for the total P is due to some error which was discovered too late to permit making a redetermination.

† Since after 3.5 weeks of incubation more than 50 per cent of the phosphorus was soluble, more rock and sulfur was added to make up a mixture with a total phosphorus content about 7 per cent.

is made available to warrant the employment of concentrated composts, it must be admitted that the data at hand do not justify an affirmative answer. The present data do prove, nevertheless, that there is a possibility that some chemical treatment may be worked out, in which a stimulation of the bacterial activities will be brought about, which will in turn speed up the sulfonation processes." The citation shows the appreciation of the difficulties and a hopeful vision. The achievement of a concentrated compost is an accomplished fact, although the incubation period is still somewhat long. In experiments yet to be reported, another step forward has been made toward the solution of the problem of making acid phosphate by the Lipman process.

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MICROBIOLOGICAL ANALYSIS OF SOIL AS AN INDEX OF
SOIL FERTILITY: IV. AMMONIA ACCUMULATION
(AMMONIFICATION)¹

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When the physiological methods for measuring the activities of soil micro-organisms were first suggested, they were looked upon as giving a biological basis for measuring soil fertility. A great amount of work has been done along this line and we are still uncertain whether these soil biological activities as determined by the present methods actually interpret soil fertility. Of these activities, the so-called ammonification, together with nitrification and nitrogen-fixation occupy the leading place. It is the purpose of this as well as the following papers to compare the various methods in use and to establish whether or not the phenomena in question can actually be used as soil biological functions. No attempt will be made to review the extensive literature, but attention will be called to some of the outstanding investigations having a direct bearing upon the problem under consideration. A detailed study of the literature on ammonification up to 1910, is given by Löhnis (34).

The measurement of ammonia formation or rather ammonia accumulation from a certain organic substance, as a source of nitrogen, usually a complex protein, is used by the soil bacteriologist for the study of four groups of phenomena which may be outlined as follows:

1. AVAILABILITY OF NITROGENOUS FERTILIZERS. Valuable information has been obtained from the study of ammonia accumulation from various organic and inorganic materials, in simple or mixed fertilizers, which can serve as an indication of the speed with which these materials will be broken down in the soil with the liberation of ammonia when used for fertilizing purposes [Lipman and associates (27, 31)]. C. B. Lipman and Burgess (22) found, however, nitrification of organic nitrogenous materials a better index of their availability than ammonia accumulation. Lathrop (20) pointed out that in the decomposition of protein materials, such as dried blood, intermediate compounds are formed which are undoubtedly beneficial to plant growth. He maintained, therefore, that ammonification is not a complete index of the availability of nitrogen in organic fertilizers. This applies, of course, to nitrification as well.

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2. COURSE AND RAPIDITY OF DECOMPOSITION OF NITROGENOUS ORGANIC MATTER. When ammonia is used as an index of the amount of organic matter decomposed, important information can be obtained as to the influence of various environmental conditions, such as moisture, aeration, physical and chemical condition of medium, presence and absence of other nitrogenous and non-nitrogenous organic and inorganic substances, influence of stimulants, etc., upon the decomposition of the organic matter. As an important example, we might mention the decomposition of manure by microorganisms, a review of which is given by Löhnis (34, p. 580). This subject has been recently taken up by Conn and Bright (9). In this case, however, especially if a pure protein is used, a clearer understanding should be had of the mechanism of ammonia formation and of the other processes, leading to the disappearance of ammonia, such as nitrate formation and ammonia assimilation by various soil bacteria, fungi and actinomycetes. For the decomposition of organic residues in the soil, containing only small amounts of nitrogen, the CO_2 production is a much better index.

3. COMPARISON OF PURE CULTURES OF MICROORGANISMS. Since ammonia can be used as an index of the rapidity of decomposition of nitrogenous organic materials, various microorganisms can thus be compared as to their ability to decompose these organic materials and relative speed of the reaction. This may not be necessarily of direct practical importance, but it is of theoretical importance, since it helps us to interpret the rôle of the various groups of soil microorganisms in the decomposition of organic matter in the soil and allows us a better insight into the activities of these various groups of microorganisms.

4. COMPARISON OF SOILS. Soils, different in nature and changed by a difference in treatment, may be compared as to their fertility, by their efficiency of accumulating ammonia from definite organic materials rich in nitrogen.

It is this last phenomenon that has been made the subject of a large part of the work on ammonification and which concerns us here. The question is whether a difference in ammonia formation from a certain organic material between two soils indicates a difference in fertility or is it merely a result of different chemical, physical, biological and merely chance factors having nothing to do with soil fertility?

The methods used in determining the ammonifying capacity of the soil are divided into two groups: First, solution methods, whereby a definite quantity of soil is introduced into a solution of a definite protein and the ammonia formed determined after a certain period of incubation and, second, soil methods in which the protein is added directly to the soil to be tested. The action of bacteria on proteins results in the formation of albumoses, peptones, amino acids, acid amides and ammonia as the final product of the processes of hydrolysis and deamination. By treating the culture (solution or soil) with MgO and boiling, the ammonia and acid-amid nitrogen are driven over as ammonia. By the aeration method only the ammonia nitrogen is determined.

The fact that soil organisms, both bacteria and fungi, are active in the breaking down of various proteins with the formation of ammonia was pointed out in 1893 by Müntz and Coudon, Marshal, and soon afterward by other investigators. Chester (8) determined the ammonifying efficiency of each species of bacteria isolated by the use of the plate and, by combining these, he expressed the zymotic ammonifying efficiency of the soil, basing this on the idea that the ammonifying efficiency of a soil is proportionate to the number of organisms and their ammonifying power.

Remy (38) suggested a simpler method: 10-gm. portions of soil were added to 100 cc. of a 1-per-cent sterile peptone solution in flasks which were then incubated at 20°C. for 4 and 8 days; the culture was then filtered and ammonia determined in 25 cc. of the filtrate by distillation with magnesium oxide. The amount of ammonia formed served as an index of peptone decomposition. Four soils were tested by this method in May, July and October. By comparing the amounts of ammonia formed in peptone solutions in 4 days by 3 soils with the corresponding crop yields, he obtained the ratios of 100/100, 144/138 and 156/163. After 8 days' incubation, however, all the soils were found to give at all times about equal amounts of ammonia. Remy concluded from these results that peptone decomposition can serve as an index of the ability of a soil to decompose nitrogenous organic matter and, therefore, of soil fertility.

By comparing the amounts of soil used for the inoculation of the peptone solution, Löhnis (32) found that an average error of 2.84 per cent is involved when 10 per cent of soil are used, 8.35 per cent with 2 per cent of soil, and 12.30 per cent with 0.1 per cent of soil. In other words, the smaller the amount of soil used for inoculation, the greater is the error involved. As a result of a study of the decomposition of various peptones as well as other proteins during various periods of the year, Löhnis came to the conclusion that the soil does not possess a constant protein decomposing power and, for the study of the influence of treatment and environmental conditions, samples should be taken as often as possible throughout a whole period. Gutzeit (17) used 1-per-cent peptone solution in soil extract and found two different soils to give the same amount of ammonia after a 5-day incubation period at 20°C. However, a distinct difference in the nitrifying capacity was obtained between the two soils. Buhrlert and Fickendey (6) used for inoculation of the peptone solution a suspension of soil in water rather than the soil itself and a temperature of incubation of 25°C. but no difference could be obtained in the ammonia-forming capacity of different soils. Lipman (23) compared the ammonifying capacity of four soils in peptone solution, using for inoculation both the soil itself and a soil suspension in water. Much higher results were obtained with the direct inoculation with soil at a temperature of 20°C. for 82 hours. However, when 0.5 per cent K_2HPO_4 , 0.2 per cent $MgSO_4$, 0.2 per cent $CaCl_2$ and sufficient sodium hydroxide to make the reaction weakly alkaline were added to the peptone solution, not only was there no difference between the two methods of inoculation, but there was no difference between the four different soils. It was suggested, therefore, that, for the study of the peptone-decomposing power of the soil, it is best to use only peptone in tap water. However, even if slight differences are obtained in favor of one soil over another when using only peptone in water, they may be due not so much to the fertility of the soil expressed by a more active bacterial flora as to the presence of larger quantities of soluble phosphates and other minerals in one soil than in another.

Vogel and Zeller (45), by incubating their cultures for 6 days at 20°, obtained no difference in the peptone-decomposing power of different soils taken at different times; if any differences were obtained, they were not greater than the error between duplicate determinations. Even slight differences in the temperature of incubation show greater differences than the physico-chemical properties of the soil itself. By replacing peptone by hornmeal greater differences were obtained due to the slower reaction.

Albert and Luther (1) found the Remy method to give valuable information on the chemico-biological processes in forest soils. Vogel (44) as well as Lemmermann and associates (21) found that various soils show little difference in ammonifying efficiency when inoculated into solutions; differences appear only when nitrification takes place. If any differences are obtained by carrying out the ammonification test according to Remy, they are chemical rather than bacteriological in nature. That the method of Remy has little justification has also been pointed out by Ritter (40) in the study of the influence of drying on the biological activities in the soil. Lipman (23, 24) found that the peptone-decomposing power of a soil is greatly influenced by the application of manure. Lipman attempted to establish a direct relationship between crop production and protein-decomposing power, using 1-per-cent

peptone, casein, albumin, but without any satisfactory results. Remy (38) replied that the decomposition of proteins by soil should not be looked upon as of deciding moment in soil fertility.

Fischer (12, 13) as well as Dzierbicki (10) pointed out that the chemical composition of the soil introduced with the soil infusion and particularly the content of phosphates has a greater influence upon ammonia formation in peptone solution than the bacterial population. Rahn (37) also suggested that differences obtained, by the solution method, between various soils, are due largely to the mineral content of the soil inoculum.

Löhnis (32, 33) was the first to modify the peptone solution by using, in place of water, an extract of soil, to which peptone or other protein material is added. Fischer (11) soon pointed out, however, that, under these conditions, the difference in ammonia formation may be due rather to the extract than to the inoculated soil.

The original Remy method was modified by Remy and Rösing (39) whereby 10 per cent of soil in the form of a soil suspension (50 gm. of soil in 250 cc. sterile physiological salt solution shaken for 5 minutes) is used for inoculation, cultures incubated for 4 days and ammonia determined in the whole culture including the soil. By using various proteins and peptones, Remy concludes that the peptone decomposition in solution is an index of the protein decomposition in the soil. In view of the fact that peptone is too rapidly decomposed in comparison with the nitrogen compounds in the soil, it is suggested to use 1 per cent of pure gelatin, 0.1 per cent each of K_2HPO_4 , $MgSO_4$ and Na_2CO_3 and 10 per cent of sterile soil extract; 0.1-0.25 gm. of soil in suspension should be used for the inoculation of 50 cc. of the cultures, which are then incubated at 20°C. for 7-8 days. Voitkevitch and Kolenev (46) obtained no greater differences between the peptone-decomposing power of five different soils than between duplicate checks of the same soil.

In view of the fact that the solution method was found unsatisfactory, Vogel (44, 45) suggested using the soil itself: adding 5 gm. of hornmeal to 500 gm. of sieved soil, mixing well, adding 12 per cent water after and placing in flasks, plugging with cotton and incubating 12 days at 23°C. By determining the organic, the total, the ammonia, and the nitrate nitrogen, Vogel demonstrated that the decomposition of hornmeal takes place in different soils with the same intensity, but the differences are found only when the ammonia is transformed into nitrates.

It is interesting to note that the predominance of certain groups of bacteria in laboratory tests using the solution method, depends upon the kind of protein used, as pointed out by Löhnis and Parr (36).

No relation between bacteriological activities in soil and in solution were found by Stevens and Withers (41, 42), the results being very variable, some bacterial soil complexes ammonifying faster in soils than in solutions and vice versa. They suggested standard methods for ammonification studies with soils, by determining the ammonifying efficiency, ammonifying inoculating power and ammonifying capacity, and believed that the results obtained by using soil as a medium were more trustworthy. Löhnis and Green (35) claimed that no necessary fundamental difference should be regarded as existing between bacteriological processes in soil and solution media.

Remy and Rösing (39) also suggested that more positive information is obtained in soil than in solution. Lipman and associates (25, 30) found, by the addition of dried blood or other nitrogenous organic matter to 100-gm. portions of soil, keeping at suitable moisture and temperature conditions for several days, that ammonia formation may serve as an index of the intensity of the decomposition processes in the soil. This method (tumbler method) has come into general use and is similar to determining the ammonifying efficiency suggested by Stevens and Withers. Lipman and Brown (29) obtained similar results by the solution and soil tests. However, the variability between the duplicate determinations and the difference in the amounts of ammonia formed from the different nitrogenous materials are of such a nature that one may question the value of the ammonification test altogether. We are particularly justified in doing that when we compare the results obtained by these

investigators from the ammonification tests, with the bacterial numbers and nitrifying capacity of the same soils. The latter two methods yield uniform results, while there is an entire lack of uniformity in the ammonification tests.

On studying the influence of seasonal variation upon ammonia formation in soil and in solution, Löhnis and Green (35) found this variation to lie on the border of experimental error between duplicate tests.

Greaves (15) found no difference in the ammonia formation of virgin and cultivated soils, although a nearly quantitative relationship was found to exist between the number of the colonies, nitrification and nitrogen fixation. In a latter study of the influence of manure on the soil, Greaves and Carter (16) found a direct relationship to exist between the bacterial numbers, ammonifying and nitrifying powers and crop production.

Brown (4, 5) found a close correlation between crop-producing and ammonifying power of the soil. Brown (3) states, in another place, that fresh soil is the most rational medium for studying the physiological activities of soil bacteria. Fresh soil permits of the greater differentiation in field soils differently treated, according to the activities of ammonifying bacteria, than air-dry soil inoculated with the soil infusion. This need not necessarily point to the value of the ammonification test, since we might conclude that it is the physical and chemical condition of the soil which account for the differences in ammonia formation rather than the bacteriological condition. Where the same air-dry soil is used for the inoculation with suspensions of various soils, we get little differentiation due to the fact that the medium has the same physical and chemical condition. This will also account for the fact that Brown found air-dry soils from particular plots inoculated with infusions of fresh soils to show greater differences than an air-dry standard soil inoculated with infusions from the various soils. Brown himself recognized that "the chemical character of the soil is of considerable moment when considering its ammonifying efficiency."

Kelley (18) found that both good and poor soils supported vigorous ammonification. Gainey (14) concludes from a study of various soil types, of different productivity, from widely different localities that there is no correlation between yield and ammonia nitrogen content. Lipman and Burgess (22) found the nitrification test a more reliable criterion than the test of ammonification. By comparing a large number of soils, they found a decided tendency toward a more or less uniform ammonifying efficiency of all the soils tested. They suggested the discarding of the ammonification method. Burgess (7) states, as a result of studies on Hawaiian soils that, as a rule, ammonification tests are not suitable in differentiating good and poor soils, although they often show differences between very poor and very good soils. A lack of correlation between ammonification studies and crop productivity has previously been pointed out by Fischer (13). Temple (43), as a result of extensive studies concludes that "The ammonification test is of little value in determining the nature of the soil micro-flora, since, (a) the soil medium largely controls the rate of ammonia production, (b) all soils are well supplied with bacteria, capable of changing proteid nitrogen to ammonia if the soil is made a suitable medium, and (c) the test as usually conducted does not take into consideration the considerable amounts of ammonia that may escape into the atmosphere or be converted to nitrate or nitrite. Ammonification can properly be used by the soil chemist as a test of soil fitness."

THEORETICAL

As seen from the brief historical review, we are still uncertain as to whether ammonification studies can be used for the interpretation of soil fertility. Some investigators (Fischer and Temple) condemn these studies as valueless in determining the nature of the soil flora. At best they are merely an indication of soil fitness, since the ammonia formation in the soil is largely controlled by the soil medium. Others (Brown) still continue to use ammonification

studies for measuring soil fertility and even attempt to predict fertility from these data. Before taking up the experimental results which will tend to clear up the subject in question, it may not be out of place to discuss first a few chemical and biological principles involved in ammonia formation from proteins in the soil or in solution inoculated with soil.

The process of ammonification is extremely complex, from the biochemical viewpoint, and is most intimately connected with the decomposition of protein materials in the soil, as pointed out by Lathrop (20). Then there are a large number of groups of microorganisms in the soil that are able to break down proteins with the formation of ammonia, some at a rapid speed, others more slowly, still others only very slowly, depending upon the nature of the organism, length of period of incubation, nature of culture medium and presence or absence of carbohydrates and other non-protein substances. A change in the physical and chemical condition of the soil will affect in a different way the activities of these different groups of soil microorganisms and the type of organic compounds formed from the decomposition of proteins. The majority of organisms developing on the common plate used for counting bacteria belong to one of four groups, viz., the spore-forming and non-spore-forming bacteria, fungi and actinomycetes, and all of these are able to form ammonia from proteins. The speed of the reaction, however, will be different for these four groups. The fungi break down proteins in the soil very rapidly and within 7-10 days 50 per cent of the nitrogen in the protein may be transformed into ammonia by pure cultures of these organisms. The actinomycetes will, in that period of time, due to their comparatively slow growth, produce only little ammonia; when, however, the analysis is made after 30-50 days, it will be found that the actinomycetes as a rule will have allowed as great an accumulation of nitrogen as the fungi if not greater. The same may be true of the two groups of bacteria. While the general impression has been that the spore-forming bacteria are the most active ammonia-forming organisms in the soil, due to their rapid development on the plate, Conn and Bright (9) found that a non-spore-forming organism will produce much more ammonia from manure than the spore formers.

Modifying the soil, as by adding organic matter, inorganic fertilizers, lime, moisture, cultivation, etc., does not modify the activities of these various groups of soil organisms to the same extent. The addition of lime, for example, to an acid soil results in a corresponding increase in the number of actinomycetes and decrease in fungi. It may result in slower, but more accumulative formation of ammonia, so that, in a brief period of incubation, the acid soil may produce more ammonia, due to the relative greater abundance of fungi, while the limed soil may give a higher ammonia formation when the period of incubation is prolonged. Then the lime treatment of an acid soil will make conditions more favorable for the activities of the nitrifying bacteria which will transform a part of the ammonia into nitrates. A number of other reactions may be brought about by the lime treatment which will affect the

amount of ammonia accumulated in the soil, when a comparatively large amount of protein has been added to the soil, under artificial laboratory conditions. To state that ammonia accumulated, under these conditions, is an index of soil fertility, with a good many results pointing the other way, is not scientifically correct.

Conditions which tend to bring about a change in the groupings of the four groups of microorganisms will also affect the amount of ammonia that will accumulate. When a small quantity of soil is introduced into a peptone solution, the rapidity of the reproduction of the various microorganisms, particularly bacteria, will bring about as rapid an increase in numbers in the flasks inoculated with a good fertile soil as in those inoculated with a poor soil. After all, the difference between the total numbers of microorganisms in a fertile and a corresponding unfertile soil may be about 3 to 1, yet it will not take very long before the flasks inoculated with the latter will contain as many bacteria as the flask inoculated with the former as pointed out by Rahn (37). This is true, of course, when the solution contains, in addition to the peptone, also the minerals necessary for the activities of the microorganisms. When however, only peptone (or any protein) solution is used, the introduction of the necessary amount of soluble minerals with the amount of soil used for inoculum will affect the rapidity of multiplication of the bacteria and, therefore, the ammonia formation. In this case we are testing not the bacterial activities in the soil, but the presence of soluble minerals, which can be accomplished much more readily by direct chemical analysis. This was recognized by Löhnis (33, 34) and Remy (39) who suggested the addition of soil extract to the culture.

Another disadvantage of the solution method consists in the fact that two of the most active protein-decomposing organisms in the soil the fungi and actinomycetes, do not have a chance to develop in the solution due to the rapid development of bacteria, rapid accumulation of ammonia and short period of incubation. Only the aerobic and the facultative anaerobic, the so-called putrefactive bacteria, develop predominantly and these, by their rapidity of multiplication, universal distribution, will readily account for the lack of differentiation of soil bacteriological activities, by the solution method. If there is any difference at all, it can readily be accounted by the difference in the chemical composition of the inoculum.

Ammonia is formed by the various groups of soil microorganisms chiefly as a waste product in the utilization of proteins as a source of energy. It is also produced, of course, in the nitrogen metabolism of the organisms, in the hydrolysis of proteins and their degradation products by enzymes and in the autolysis of the microbial cells, but to a comparatively much smaller extent. Microorganisms can use both carbohydrates and proteins as sources of energy. When there is present in the soil, in addition to proteins, a sufficient supply of carbohydrates, the microorganisms will use the latter as a source of energy and will attack the proteins only in so far as they need nitrogen for their

metabolism, in this case very little ammonia will accumulate in the soil and, even if some is produced, it may rapidly be used up by various other microorganisms as a source of energy. This will account for the small amount of ammonia formed or accumulated in the soil, when dextrose or other available carbohydrates and even straw are added to the soil, as shown by Lipman and associates (28), Waksman (48) and others. But, when there is an insufficient amount of available carbohydrates in the soil, the microorganisms will attack the proteins not only as a source of nitrogen, but as a source of carbon, which yields the necessary energy. The proteins contain about 50 per cent of carbon and about 10 to 15 per cent of nitrogen. The microorganisms require a great deal more carbon than nitrogen for their metabolism. The carbon will then be rapidly oxidized, while the excess of nitrogen will be left in the medium as a waste product, in the form of ammonia.

According to Kruse (19) the weight of the fungus body will be, under the most optimum conditions, about one-third of the sugar consumed. Protein or protein-degradation products are used much less economically, so that, for 1.5 gm. of peptone consumed there is a yield of about 0.162 gm. of fungous mycelium which will contain about 12-15 mgm. of nitrogen. One and one-half gram of peptone may contain about 200 mgm. of nitrogen, so that 90 per cent of the nitrogen will be left as a waste product in the form of ammonia, with peptone as the only source of energy. The utilization of available energy by bacteria is even less efficient. *B. proteus*, for example, consumed, in a period of 30 days, out of 793 mgm. available nitrogen only 97 mgm., when it had to derive its energy from proteins. Urea bacteria, according to Kruse (citing the work of Miquel), convert 4000 parts of urea to build up 1 part of body substance. The addition of a small amount of available carbohydrate will check for a short period of time the ammonia accumulation, although it will result in an increased microbial flora. After all the carbohydrate is used up, the microorganisms will attack the proteins present and may even produce larger quantities of ammonia in the soil than when no carbohydrate has been added, as pointed out by the author elsewhere (47). However, when the amount of carbohydrate added is large, it will prevent the ammonia accumulation for some time and perhaps for the whole length of the usually short period under which the laboratory tests are carried out.

EXPERIMENTAL

The soils used in the following investigations were obtained from a series of plots laid out by this department 15 years ago and receiving the same fertilizer treatment. A careful record of treatments and crop yields was kept and has been discussed by Lipman and Blair (26) and by the author (48). These plots have been so modified by the continuous treatment as to establish definite microbiological differences in the study of numbers of microorganisms, as pointed out in the previous paper (48) and as will be shown in the following paper in the study of the nitrifying capacity of these plots.

The plots seem to be, therefore, well suited for finding out whether ammonification studies by one or all the methods in use can indicate differences in soil fertility. The treatment of the plots, carbon and nitrogen content at 1917 and total crop yield for the last 14 years are reported in table 7. Both the solution methods and soil methods were employed, the latter in connection with the study of nitrification, soil reaction and numbers of microorganisms.

AMMONIA FORMATION IN SOLUTION

The solution method was carried out as follows: 100 cc. portions of 1-per cent solutions of Bacto-peptone, casein or Gold Label gelatin in distilled water with or without 0.05 per cent K_2HPO_4 , were placed in 250-cc. Erlenmeyer flasks, plugged with cotton and sterilized, for 15 minutes at 15 lbs. pressure. The casein was previously dissolved in 0.1 *N* NaOH, then adjusted with 0.1 *N* HCL to a reaction of pH 6.5-7.0. The flasks were inoculated with 10 cc. of a 10-per-cent suspension of the soil shaken for 5 minutes, unless otherwise stated, and incubated at 28-30°C. for 3-7 days. The whole culture was then transferred into copper flasks and the ammonia determined by distillation with MgO, in the usual manner. Two to four flasks were used for each determination, but only the averages are given, in view of the fact that the duplicate determinations checked up fairly well in the majority of cases.

The results presented in table 1 point out clearly the fact that the amount of inoculum plays an important part, when only a 1-per cent peptone solution is used. The influence of inoculum was studied in the presence of 0.05 per cent K_2HPO_4 in order to find out whether the differences in ammonia formation are due to the specific microbiological flora of the plots or to the larger supply of soluble phosphates and potassium salts in the more fertile plots than in the less fertile plots. For this purpose only 5A and 7A were used as representing the most productive and least productive plots, without the interference of the lime factor.

Table 2 shows that much smaller differences are found between the fertile and non-fertile plot, when K_2HPO_4 is used. Although in most cases the soil from the fertile plot (5A) gave higher ammonia formation than the soil from the unfertile plot (7A), the reverse held true in one case.

No period seems to show any advantage over any other in bringing out differences in ammonia formation important enough for differentiating the more fertile from the less fertile plot, although in all cases the amounts of ammonia formed by the former was somewhat higher than those formed by the latter.

A series of plots were next studied for the influences of fertility upon ammonia formation by the respective soils in solution of various proteins. Table 3 shows that the limed plots had in all cases a slightly higher ammonia-content than the non-limed plots; practically no correlation is found between the fertility of the plots and ammonia formation in solution. Plots 5A and 5B are the most fertile, 7A and 11A had practically no crops due to the excessive

acidity, 7B and 11B were intermediate. Plot 11A which is so acid as not to allow any crop growth, gave in the peptone solutions a greater amount of ammonia than 5A; 7B which received nothing but lime for the last 15 years gave, both in the peptone and casein solutions, higher amounts of ammonia than 5B, the heavily manured plot.

TABLE 1

*The influence of amount of inoculum upon ammonia formation in 100 cc. of a 1-per-cent peptone solution**

PLOT NUMBER	NH ₃ -N FORMED WITH VARIOUS AMOUNTS OF INOCULUM		
	0.01 gm. of soil	0.1 gm. of soil	1 gm. of soil
	mgm.	mgm.	mgm.
5A	42.28	46.78	55.72
5B	40.32	48.02	55.58
7A	30.80	41.86	44.94
7B	29.96	40.04	43.82
11A	32.34	41.30	49.56
11B	35.15	45.64	52.08

* Cultures incubated 3 days. Soil sampled January 28, 1922.

TABLE 2

The influence of amount of inoculum and period of incubation upon ammonia formation, in 100 cc. of 1-per-cent peptone solution with 0.05 per cent of K₃HPO₄

PROTEIN SOURCE	PERIOD OF INCUBATION	AMOUNT OF INOCULUM	NH ₃ -N FORMATION	
			5A	7A
	days	gm.	mgm.	mgm.
1-per-cent peptone solution	1	1.0	11.25	10.28
	2	1.0	55.14	51.27
	3	0.1	76.16	70.49
	3	1.0	78.87	76.53
	3	10.0	93.94	77.07
	7	1.0	103.18	100.38
1-per-cent casein solution	7	0.1	94.50	89.18
	7	1.0	99.96	93.94
	7	10.0	89.04	95.76

The same experiment was repeated with the soil from 4 distinct plots 5A, 7A, 5B, 7B, using small and large amounts of inoculum, in the presence and absence of K₃HPO₄.

It is interesting to note (table 4) that in all cases there is an increase in ammonia formation with an increase in the amount of inoculum used. Plot 5A, which is a very fertile soil, gave in the absence of phosphates higher amounts of ammonia than 7A which lacks in fertility; in the presence of phosphate, the differences are very small, particularly with the larger amounts

of inoculum. Plot 5B, however, which received the same treatment as 5A in addition to lime, gave lower amounts of ammonia both with small and large inoculum, in the presence and in the absence of phosphates.

The results obtained from the above experiment would lead us to *condemn completely the study of ammonification in solution and any attempts to correlate soil fertility with any differences in ammonia produced as fultile.*

TABLE 3
*Formation of ammonia in 100-cc. portions of 1-per-cent solutions of various nitrogenous substances**

PLOT NUMBER	PEPTONE		CASEIN		GELATIN
	No phosphate†	0.05 per cent K_2HPO_4 ‡	No phosphate†	0.05 per cent K_2HPO_4 ‡	No phosphate†
	mgm. NH_3-N	mgm. NH_3-N	mgm. NH_3-N	mgm. NH_3-N	mgm. NH_3-N
5A	78.87	82.7	77.9	89.96	47.1
5B	83.02	83.9	78.3	89.54	58.1
7A	76.53	84.2	78.8	83.94	42.5
7B	88.5	89.26	82.4	90.10	47.7
9A				86.60	
11A	81.29	88.5	77.7	84.50	41.2
11B	87.69	88.7	78.7	84.64	42.4
19A	82.67			85.34	42.4

* One gram of soil (5 cc. of a 20-per-cent suspension) was used for inoculation.

† The reaction of the protein solutions was adjusted to pH = 7.0. Peptone cultures incubated for 4 days, casein and gelatin for 6 days. 10 cc. of 10-per-cent soil suspension was used for inoculation.

‡ The cultures with the phosphate were prepared a week after the casein cultures without the phosphate.

TABLE 4
Ammonia formation in 100 cc. portions of solutions as influenced by amount of inoculum and presence of K_2HPO_4

PLOT NUMBER	1-PER-CENT PEPTONE			1-PER-CENT PEPTONE AND 0.5 PER CENT K_2HPO_4		
	0.01 gm. soil	0.1 gm. soil	2 gm. soil	0.01 gm. soil	0.1 gm. soil	2 gm. soil
	mgm. NH_3-N	mgm. NH_3-N	mgm. NH_3-N	mgm. NH_3-N	mgm. NH_3-N	mgm. NH_3-N
5A	80.08	80.08	87.64	76.16	79.38	86.80
7A	67.76	76.72	83.02	71.40	78.12	84.84
5B		78.12	85.96		78.96	84.28
7B		81.62	88.76		85.12	88.62

AMMONIA FORMATION IN THE SOIL

The extensive amount of work done on the formation of ammonia, by the use of fresh soil, and the claims put forth (Brown) that these results are parallel with crop production might lead one to look here for a method differentiating soils of different fertility. For these experiments the fresh soil

was placed, as soon after sampling as possible, in 100-gm. portions into tumblers. One-gram portions of dried blood (11.50 per cent nitrogen) were well mixed with the soil. The tumblers were covered with glass and placed in the incubator, 28–30°C. for 7 days. The moisture was kept up to optimum by addition of distilled water. The ammonia was then determined by the usual method. In some cases, 200-gm. portions of soil with 2 gm. of dried blood were placed in the tumblers, and, in addition to ammonia determinations, pH and nitrate determinations were made. Duplicates or triplicates were always made, but only averages are recorded, since duplicates usually agreed well.

The results presented in table 5 were obtained at three different periods when samplings of soil were made, January 28, April 11 and May 22, 1922. In the case of the winter sampling, the tumblers were allowed to remain for one or two days uncovered, till the moisture was reduced to the optimum.

TABLE 5
Ammonia formation in 100 gm. fresh soil

PLOT NUMBER	JANUARY 28	APRIL 11	MAY 22
	<i>mgm. NH₃-N</i>	<i>mgm. NH₃-N</i>	<i>mgm. NH₃-N</i>
5A	30.87	50.7	45.22
7A	32.76	38.1	38.08
9A	34.02
11A	38.50	44.1	44.24
19A	39.34
5B	18.41	41.9	42.00
7B	15.26	45.2	40.04
11B	28.49	56.7	47.46

The results presented in table 5 indicate that not only is the use of ammonia formation in the soil as an index of soil fertility unjustified but that there is no soil differentiation at all. The reason for the low results in the January test is probably the insufficient aeration of the soil which was placed in the tumblers in a saturated condition.

The results become more interesting when we compare the ammonia formation at different periods. Table 6 shows that ammonia accumulation in the soil was higher in the acid and less fertile plots. This is due not so much to their greater capacity of forming ammonia as to their lower capacity of transforming the ammonia into nitrates, which is an entirely different story. This question will be discussed at length in the following paper of this series.

It becomes at once apparent, from table 6 that the amount of ammonia accumulated in the soil, after a certain period of time, is not at all an index of the fertility of the soil, but may merely indicate how incapable a certain soil is of transforming that nitrogen into nitrates.

The results here reported represent only a few experiments selected at random from a large number of investigations on the relation of ammonia for-

TABLE 6

Course of ammonia accumulation and nitrate formation in 100 gm. of soil and 1 per cent of dried blood

PLOT NUMBER	NH ₃ -N AFTER				NO ₃ -N AFTER 27 DAYS	TOTAL NH ₃ AND NO ₃ -N
	3 days	7 days	15 days	27 days		
	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
5A	11.69	45.22	77.56	69.80	42.2	112.00
7A	11.90	38.08	63.70	72.50	4.8	77.30
9A	9.38	34.02	70.42	78.96	7.6	86.56
11A	14.56	44.24	76.16	90.30	5.0	95.80
19A	11.34	39.34	74.06	88.20	8.8	97.00
5B	13.16	42.00	58.10	49.70	43.2	92.90
7B	12.04	40.04	53.20	57.80	5.4	63.20
11B	10.50	47.46	59.78	69.20	4.9	74.10

TABLE 7

Biological analysis of soil as an index of soil fertility

PLOT NUMBER	NH ₃ -N*, PEPTONE SOLUTION	NH ₃ -N†, CASEIN SOLUTION	NH ₃ -N‡ in soil	NO ₃ -N§ in soil, D.B.	NO ₃ -N§ in soil (NH ₄) ₂ SO ₄	BACTERIAL NUMBERS	CROP YIELDS PER ACRE FOR 14 YEARS	REACTION	SOIL TREATMENT	TOTAL CARBON	TOTAL NITROGEN
	mgm.	mgm.	mgm.	mgm.	mgm.	mil-lions ¶	lbs.	pH		per cent	per cent
5A	78.87	99.96	45.22	42.2	4.30	17.84	60,541	5.4	Minerals, 16 tons of cow manure	1.44	0.1185
7A	76.53	93.94	38.08	4.8	0.56	7.14	15,295	4.6	No fertilizer	0.93	0.0785
9A	87.41	96.60	34.02	7.6	4.80	18.25	50,488	5.9	320 lbs. NaNO ₃ , minerals	1.13	0.0975
11A	81.29	94.50	44.24	5.0	0.51	4.80	38,731	4.4	(NH ₄) ₂ SO ₄ equivalent to 320 lbs. NaNO ₃ , minerals	1.21	0.0904
19A	82.67	95.34	39.34	8.8	1.56	8.20	27,227	5.5	Minerals only	1.01	0.0872
5B	83.02	99.54	42.00	43.2	12.40	13.40	55,034	6.4	Manure, minerals, lime	1.42	0.1143
7B	89.26	100.10	40.04	5.4	7.20	11.70	27,239	6.3	Lime only	1.02	0.0821
11B	87.67	94.64	47.46	4.9	3.80	12.74	53,826	5.7	(NH ₄) ₂ SO ₄ , minerals, lime	0.06	0.0819

* Peptone cultures (10 cc. of 10-per-cent suspension of soil in water added to 100 cc. of 1-per-cent peptone and 0.05-per-cent K₂HPO₄ solution) incubated for 3 days.

† Casein cultures (same medium) incubated 7 days.

‡ NH₃-N in soil signifies ammonia formed in 7 days from 1 gm. of dried blood in 100 gm. of soil.

§ NO₃-N signifies nitrate nitrogen formed in 100 gm. of soil from 1 gm. of dried blood (D. B.) or from 30 mgm. of N as ammonium sulfate, in 30 days.

|| Fertilizer quantities and yields are given on acre basis. The B plots received 1 ton of ground limestone per acre in 1908, 2 tons in 1913, and 2 tons in 1918.

¶ Numbers show total number of microorganisms developing in 7 days, at 28-30°, on albumen agar plate, in millions per gram of soil.

** For this apparent lack of correlation see discussion on p. 62 of text.

mation to soil conditions and crop productivity. Results have been negative, with very few indications of differentiation. This is particularly striking in view of the fact that a definite correlation is found between soil productivity and numbers of microorganisms as shown previously and the nitrifying capacity of the soil, as will be shown in a later paper. A complete summary of results obtained on the ammonification on May 22 as correlated with some other soil biological phenomena and crop yields are given in table 7.

There is, a definite correlation between numbers of microorganisms and crop yields, also between these and nitrifying capacity for the unlimed plots only. The bacterial numbers for plot 9A run lower on the average than those of 5A as shown from the yearly average given by the author (48, p. 338). The large differences between the nitrification of dried blood and ammonium sulfate is limed and unlimed soils and apparent lack of correlation with bacterial numbers and crop yields is due entirely to the inadequacy of the methods used as present for measuring nitrification, as will be shown in the following paper. The complete lack of correlation between ammonification in soil and in solution and crop productivity bears out the statement previously made as to the unreliability of this method as a soil bacteriological function.

The results obtained from measuring ammonia accumulation in the soil, which is only a product resulting from the artificial development of a heterogeneous group of organisms some of which may or may not be inhabitants of the particular soil, do not correlate with soil productivity and other soil bacteriological activities as indicated by number of microorganisms, nitrifying capacity, carbon dioxide-producing capacity, etc. We may still, however, be able to obtain from the rapidity of decomposition of proteins as indicated by the disappearance of the proteins from the soil, either as a result of the activities of the microorganisms themselves or their enzymes, a reliable index of soil fertility.

SUMMARY

1. Ammonification in solution cannot be used for soil differentiation, for the following reasons:

a. Of the three large groups of soil microorganisms, only the bacteria develop in solution, while the fungi and actinomycetes do not usually develop because of the nature of the medium and period of incubation.

b. Of the bacteria, those which develop are only the so-called putrefactive forms or those that are able to grow rapidly in liquid media, with peptone or other protein or protein derivative, as the only source of carbon and nitrogen. These forms are usually present in all soils, independent of the fertility of the particular soil.

c. Even where differentiation between soils is obtained it may be due more to the presence or absence of certain minerals in the soil introduced as an inoculum rather than to the particular soil flora.

2. Actually no definite correlation was found between ammonia formation in solution on the one hand and crop production, bacterial numbers and nitrification on the other.

3. Ammonification in soil cannot be used as an index of soil fertility:

a. All soils contain various groups of microorganisms that are able to break down complex proteins, with the formation of ammonia.

b. The final amount of ammonia accumulated in the soil is a resultant of a number of factors, among which are the nature of the protein used in carrying out the test, the presence or absence of available carbohydrates, the initial reaction of the soil and buffer content of the soil, the rapidity with which the ammonia is transformed into nitrates, which depends upon the initial reaction of the soil, buffer content, etc., and, the loss of ammonia into the atmosphere.

4. Ammonification can be used only in comparing the activities of specific cultures of microorganisms under controlled conditions and in studying the course of rapidity of decomposition of organic matter. It cannot be used in determining the nature of soil fertility or soil micro-flora.

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The first of these is the fact that the United States is a young nation, and its history is therefore a history of growth and development. The second is the fact that the United States is a large nation, and its history is therefore a history of expansion and conquest. The third is the fact that the United States is a diverse nation, and its history is therefore a history of conflict and compromise. The fourth is the fact that the United States is a nation of immigrants, and its history is therefore a history of assimilation and integration. The fifth is the fact that the United States is a nation of pioneers, and its history is therefore a history of exploration and discovery. The sixth is the fact that the United States is a nation of entrepreneurs, and its history is therefore a history of innovation and invention. The seventh is the fact that the United States is a nation of reformers, and its history is therefore a history of social and political change. The eighth is the fact that the United States is a nation of idealists, and its history is therefore a history of high aspirations and noble goals. The ninth is the fact that the United States is a nation of pragmatists, and its history is therefore a history of practical solutions and effective action. The tenth is the fact that the United States is a nation of optimists, and its history is therefore a history of hope and faith in the future.

The history of the United States is a story of a nation that has grown from a small colony to a great power. It is a story of a nation that has expanded its territory and influence across the continent and around the world. It is a story of a nation that has fought for its freedom and independence, and for the rights of its citizens. It is a story of a nation that has made great contributions to the world in the fields of science, art, and industry. It is a story of a nation that has faced many challenges and difficulties, but has always emerged stronger and more united. The history of the United States is a story of a nation that is full of life and energy, and that is always moving forward.

ON THE EIGHTIETH ANNIVERSARY OF THE BIRTH OF
DR. PAUL WAGNER

TRANSLATED BY THE COURTESY OF
H. G. HUSTON
Polask Syndicate

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On March 7 the noted German agricultural chemist and investigator, Dr. Paul Wagner of Darmstadt, will complete his eightieth year, at which time his numerous friends and pupils in Germany will hold a celebration at Darmstadt in his honor.

Fifty years ago, Doctor Wagner became Director of the Experiment Station at Darmstadt, which had just been founded, and has since won for this institution a world renown, through his investigations on plant foods. Doctor Wagner certainly deserves great commendation for having, with the help of his own method of pot experiments, substantially extended, and firmly established the foundation for the use of commercial fertilizers.

He was the first to recognize and correctly estimate the fertilizing effect of Thomas Phosphate or basic slag.

By a steady improvement in the methods of fertilizer experiments in the field, he succeeded in making of these field experiments a practical means of exact investigation.

Doctor Wagner, furthermore, has clearly shown to the practical farmer the results of his investigations in the vegetation house, field, and laboratory. He has done this in articles which are easily understood and in inspiring lectures and in this way has contributed in an enormous degree toward the proper use of commercial fertilizers in agriculture.

Here in the United States, many of Doctor Wagner's articles are known and have been translated or summarized by numerous writers to the great advantage of American agriculture.